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Aberrant Spermatogenesis in Hybrid Progeny of Sub-Species of the Boll Weevil (Coleoptera: Curculionidae).

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ABERRANT SPERMATOGENESIS IN HYBRID PROGENY OF SUB-SPECIES OF THE BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE)

A Dissertation

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ABSTRACT

Studies were undertaken to ascertain the mechanism(s) involved in the hybrid sterility found in the F_1 males of the cross between Anthonomus grandis thurberiae males and Anthonomus grandis grandis females. It was found that the entire process of spermatogenesis was disrupted and very chaotic in the hybrid males. Several reasons are discussed that may account for this condition.

The ultrastructure of the sperm was studied in detail by electron microscopy. The sperm possesses an acrosome, nucleus, 2 or perhaps 3 nebenkern, and 2 structures of fibrillar material which may function as supporting elements of the axial filament. The axial filament possesses the 9 + 9 + 2 arrangement of microtubules and appears to function as an undulating membrane. The nebenkern, supporting elements, and axial filament extend from the base of the nucleus to essentially the end of the tail.

The process of sperm transfer was also investigated. It is postulated that the sperm migrate up the spermathecal duct to the spermatheca in response to the secretion of the spermathecal gland which activates the sperm. This spermathecal secretion also maintains the fertilizing capacity of the sperm. When a second mating occurs 4 days after the first, 80% of the sperm from the first mating are displaced

from the spermatheca. This displacement is due solely to the flow of spermathecal gland secretions from the spermatheca.

INTRODUCTION

Around 1890 the boll weevil entered the United States from Mexico. Since that time, it has spread throughout most of the cotton producing areas of the southern United States. Pierce (1913) recognized a variety from Arizona which had been collected from the wild cotton, Gossypium thurberi (Todaro). He described it as Anthonomus grandis var. thurberiae. The boll weevil, Anthonomus grandis Boheman, was originally described by Boheman (1843) from a specimen from Veracruz, Mexico.

Warner (1966) investigated external morphological characters by which these may be distinguished. She found 3 different external morphological characters that may be used to separate the populations of weevils: 1) setae of the pronotum, 2) sculpture of the metepisternum, 3) shape and sculpture of the scutellum. On the basis of these characters she found she could separate the weevils into 3 different populations. She, therefore, suggested using the original name, Anthonomus grandis, for the intermediate form, in which the holotype of grandis is more appropriately placed. Intermediate forms are also found in western Mexico, Central America, Cuba and Baja California. The weevils which infest the cultivated cotton in the southeastern United States are Anthonomus grandis grandis. This

form is also found on the northern coast of South America. Those which infest the wild cotton (Gossypium thurberi) in Arizona and parts of western Mexico are Anthonomus grandis thurberiae.

The names used by Warner are confusing. The type specimen is an intermediate, but from a taxonomic standpoint should be the nominate form and bear the name Anthonomus grandis grandis. If there are really 3 subspecies, the common boll weevil subspecies must be renamed. For the purposes of this study, the names used by Warner will be employed. Their taxonomic status remains to be ascertained.

Newsom (unpublished) has shown that varying degrees of reproductive isolation based on inability of hybrid males to produce sperm that move into the spermathecae of females to which they were mated occur among these subspecies. This study was undertaken to ascertain the mechanisms involved in partial sterility of the hybrids. Thus, it was also necessary to investigate the morphology of the sperm and the process of spermatogenesis as well as the mechanism involved in the transfer of sperm from the male to the female.

SURVEY OF LITERATURE

Burke (1959) was the first to study the reproductive biology of the boll weevil. He described the morphology of the reproductive systems of both males and females. He described the reproductive organs of the male boll weevil as consisting of a pair of testes and their associated ducts and glands (Figure 1). Each testis is divided into 2 separate disk-shaped lobes. Each lobe of the testis is scalloped around the edge and is divided by septa into 10 or 11 wedge-shaped testicular follicles. A narrow duct arises from the inside center of each lobe and all unite to form a larger duct, the vas deferens. A short distance below this union the vas deferens enlarges to form a seminal vesicle, which becomes distended when filled with stored sperm. A pair of accessory glands arise from the sides of the vas deferens at the lower end of the seminal vesicle. The vas deferens unite to form an unpaired ejaculatory duct. The ejaculatory duct decreases abruptly in diameter before entering the muscular sheath between the apices of the aedeagal apodemes. This duct extends through the muscular sheath to open as the gonopore in the distal end of the sheath. The aedeagus of the boll weevil is a somewhat flattened, sclerotized tube. This structure, with the enclosed membranous endophallus, is exserted during

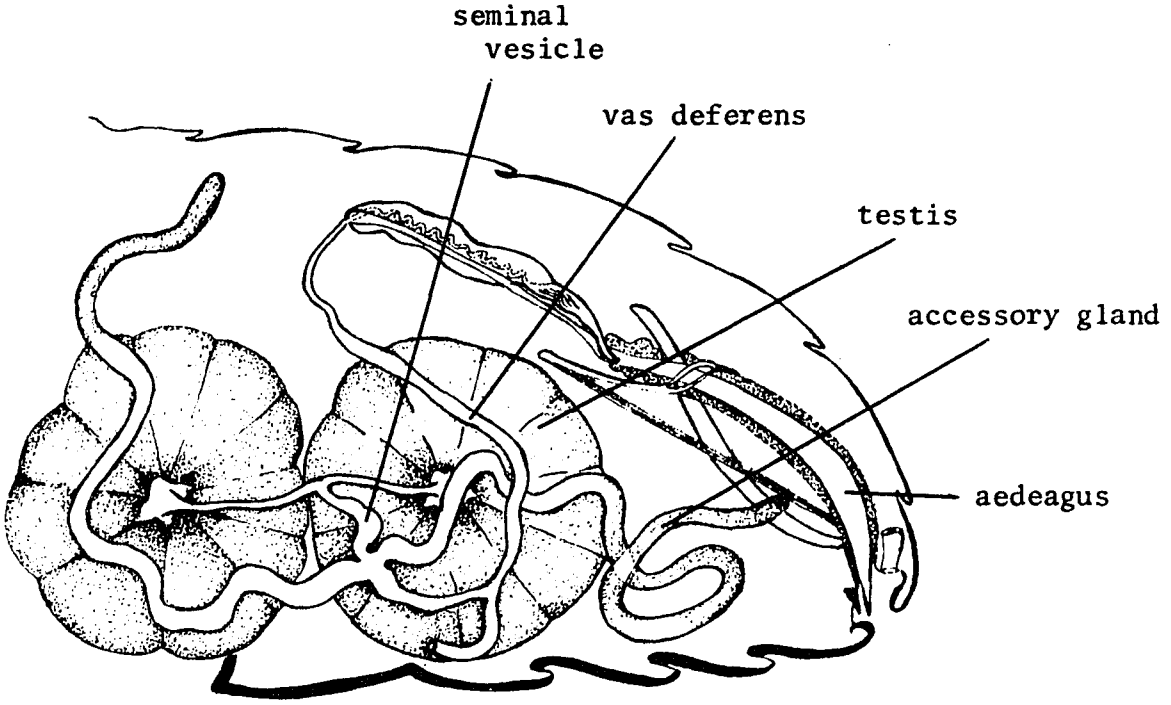

Figure 1. Male reproductive system (adapted from Burke, 1959)

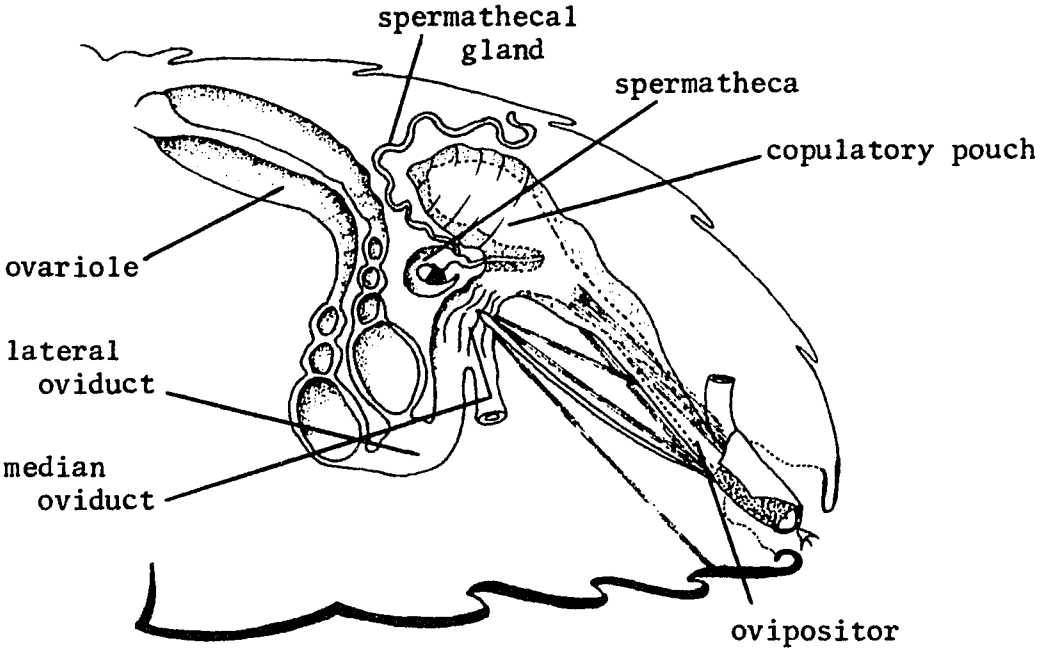

Figure 2. Female reproductive system (adapted from Burke, 1959)

copulation and transfers sperm from the male to the copulatory pouch of the female.

Burke (1959) described the female boll weevil as possessing a pair of ovarioles forming the ovary located on each side of the abdomen (Figure 2). Each pair of ovarioles opens into the widened anterior end, or calyx, of a lateral oviduct. The paired lateral oviducts unite to form the common oviduct. The common oviduct is slightly darker in color than either the lateral oviducts or the vagina. It opens into the vagina immediately behind the posterior end of the expanded copulatory pouch. The vagina extends into the ovipositor. The ovipositor is exserted during oviposition. A prominent C-shaped sclerotized spermatheca lies beneath the copulatory pouch. A slender spermathecal duct extends from the end of the enlarged portion of the spermatheca and enters the muscular tissue at the junction of the copulatory pouch and common oviduct. The long spermathecal gland opens into the spermatheca near the entrance of the spermathecal duct.

Ermert (1970) studied the spermathecal gland using both the light and electron microscope. Her study showed that it is a simple tubular gland made up of one or more layers of secretory cells around a central lumen. She concluded that the secretion is principally mucopolysaccharides of a neutral nature.

In a histological study of the boll weevil,

Chadbourne (1961) found that the male accessory glands were tubes consisting of closely nucleated epithelial cells with the viscid substance in the center being highly eosinophilic.

McLaughlin and Lusk (1967), with a light microscope, investigated the growth and cellular differentiation of testes and ovaries from the larval through the pupal stages of the boll weevil. They found that mitotic divisions of spermatogonia and oogonia occurred during larval development and the male germ cells prepared for meiotic division during the prepupal stage. Metamorphosis into adult structures could be correlated with a change in the testes from mitotic multiplication to meiotic maturation.

Chang and Riemann (1967) studied the time sequence of spermatogenesis using H^3-thymidine. They found that the spermatocytes required 10 days to mature into sperm, when measured from the period of premeiotic DNA synthesis. Of the 10 days, 4 were required to reach prophase I, less than 1 was spent in meiotic divisions, and more than 5 were spent in spermiogenesis.

In an investigation of the damage to the testes and the recovery of fertility in boll weevils fed chemosterilants, Reinecke et al. (1969) described the histology of the normal testis as seen by light microscopy. This study did not include any electron microscopy, however, the authors mapped the locations of the various stages of spermatogenesis and spermiogenesis within a testicular

lobe (Figures 3 and 4).

Lue et al. (1973) studied the karology of the boll weevil. They utilized both germinal and somatic tissue and established a diploid chromosome number of 22. They were able to classify the 11 pairs of chromosomes into 3 morphological groups.

Newsom (unpublished) investigated the possibility of reproductive isolation among crosses of the thurberia weevil, Anthonomus grandis thurberiae, and populations of the boll weevil, Anthonomus grandis grandis. He demonstrated that F_1 males of crosses between thurberia weevils and boll weevils showed varying degrees of sterility as indicated by inability of sperm to reach the spermathecae of females to which they were mated. However, sperm produced by hybrid males was capable of fertilizing eggs as long as intermittent mating was allowed.

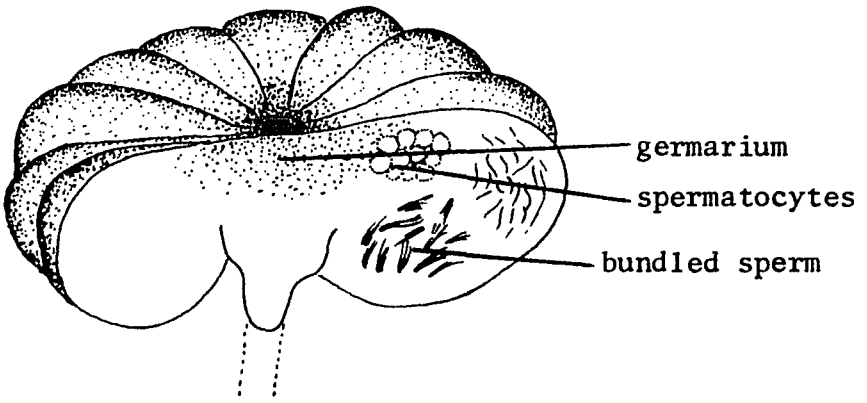

Figure 3. Three-dimensional drawing of testis lobe (adapted from Reinecke et al., 1968)

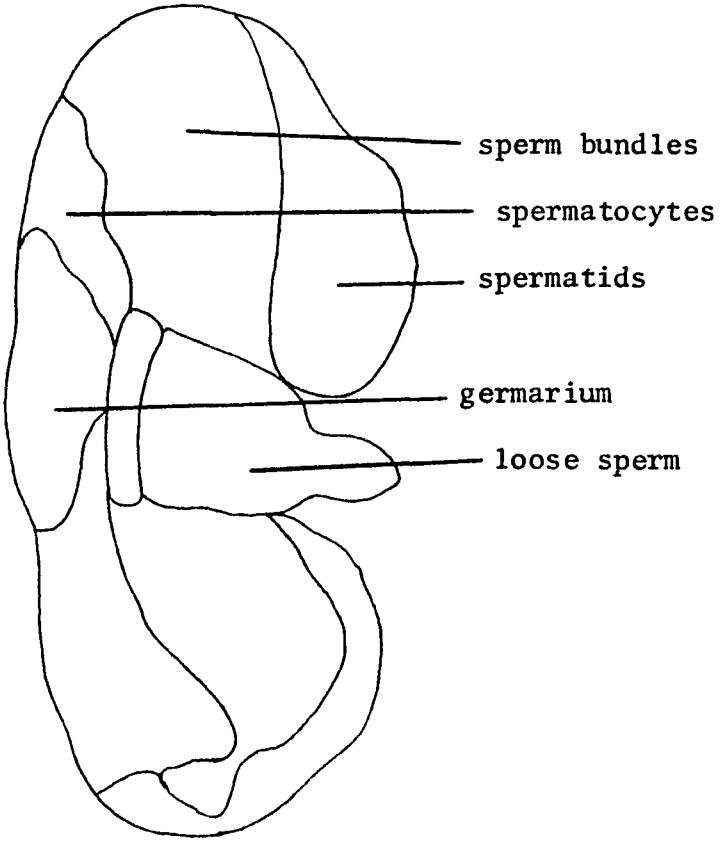

Figure 4. Interpretive drawing showing various areas of spermatogenic activity (adapted from Reinecke et al., 1969)

METHODS AND MATERIALS

Two strains of Anthonomus grandis grandis (boll weevil) were used in this study. One strain was acquired from the Boll Weevil Research Laboratory, State College, ARS, USDA, Mississippi. They were subsequently subcultured at the Cotton Insects Physiology Investigations Laboratory, ARS, USDA, Baton Rouge, Louisiana. These weevils were derived originally from an inbred Texas A & M culture established from weevils collected from cotton fields in northeastern Mexico in 1957. This strain constitutes the Mexico strain used in this study.

The second strain used was a Louisiana strain maintained in the laboratories of the Department of Entomology, Louisiana State University, Baton Rouge, Louisiana. Endrin resistant weevils were collected from the cotton fields of the Mobley Farm near Simmesport and from Cooter Point on the Tensas River near St. Joseph in Louisiana. These 2 populations were subsequently combined and designated as the MCP strain (Louisiana strain). It has been maintained in the laboratory since the mid 1950's.

The Anthonomus grandis thurberiae (thurberia weevil) were obtained from Dr. Robert E. Fye, ARS, USDA, Cotton Insects Biological Control Investigations, in Tucson, Arizona. They were shipped to Baton Rouge as diapausing adults in

the dried bolls of Gossypium thurberi. The weevils were removed from the bolls and held at 35°C under constant light until diapause was terminated. These weevils constitute the thurberia weevils used in this study.

F_1 hybrids were obtained by mating thurberia weevil males with virgin females from the Mexico and Louisiana strains. When the F_1 adults emerged they were sexed and held separate. These F_1 hybrids are referred to in this study as the Mexico hybrid and Louisiana hybrid respectively.

The weevils were reared by the methods of Earle et al. (1970). When virgins were required, adults less than 24 hours old were removed from cells in the larval diet, sexed and held in separate containers.

Sperm displacement was investigated by mating virgin females with fertile males followed 4 days later by mating with males sterilized by exposure to 10,000 r of gamma radiation from a cobalt 60 source. The eggs were collected and placed on moistened, black filter paper in a Petri dish and the hatch was checked daily. All females were retained until they ceased laying eggs. A control group of virgin females was mated with fertile males and held until they also ceased laying eggs. When the control and experimental females ceased egg production, they were dissected to ascertain if any sperm remained in the spermatheca. Sterility of the irradiated males was confirmed by mating them with

virgin females and observing egg hatch. In all cases irradiated males were found to be completely sterile. All the females in this study of sperm displacement were held in individual plastic medicine cups and fed freshly prepared synthetic diet daily.

The process of sperm transfer was studied by mating virgin females and removing the reproductive system and examining it with an A-O Phase-Contrast Microscope.

The effect of the male accessory gland secretion on sperm motility was studied by removing from the male the seminal vesicles, which were distended with sperm, and preparing suspensions in physiological saline. The physiological saline consisted of 1% sodium chloride, 0.3% calcium chloride, and 0.1% potassium chloride. Suspensions of the accessory glands were made my macerating the glands in small amounts of saline in a depression slide. One drop of the accessory gland suspension was placed in a sperm suspension and thoroughly mixed. One drop of this mixture was then placed on a microscope slide and covered with a coverslip. One drop of the sperm suspension without the accessory glands was placed on the same slide and covered with a coverslip. These two types of sperm suspensions were examined for motility with a phase-contrast microscope. An independent assessment of motility was made by a second observer. The effect of the hybrid accessory glands on normal sperm was studied in the same manner.

A determination of the protein in the accessory glands of the male was performed according to the procedure of Goa (1953). A standard curve was run using serum bovine albumin in concentrations of 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, and 2.0 mg/ml. The standard curve is shown in Figure 5. The accessory glands were prepared for analysis by dissecting them from the males and placing them in saline. The opaque and hyaline glands were placed in separate beakers in 1.5 ml of saline and refrigerated overnight. The glands were then macerated with a tissue homogenizer and centrifuged. The protein analysis was then conducted on the supernatant. The optical density was read on a Beckman DB Spectrophotometer at 330 μm.

The investigation into the role of the spermathecal gland secretion was performed by preparing a sperm suspension in saline. A spermatheca was then dissected from a virgin female, placed in a drop of the sperm suspension on a slide and then broken open. This preparation was examined with a phase contrast microscope.

The material for study with the light microscope was dissected in physiological saline and fixed overnight in Kahle's fixative. The tissue of the female reproductive system was dehydrated in graded concentrations of ethanol, cleared in xylene and embedded in Tissue Mat® (56.5°C). It was sectioned at 6μ with an AO Spencer "820" Microtome. The sections were then stained with Ehrlich's haematoxylin.

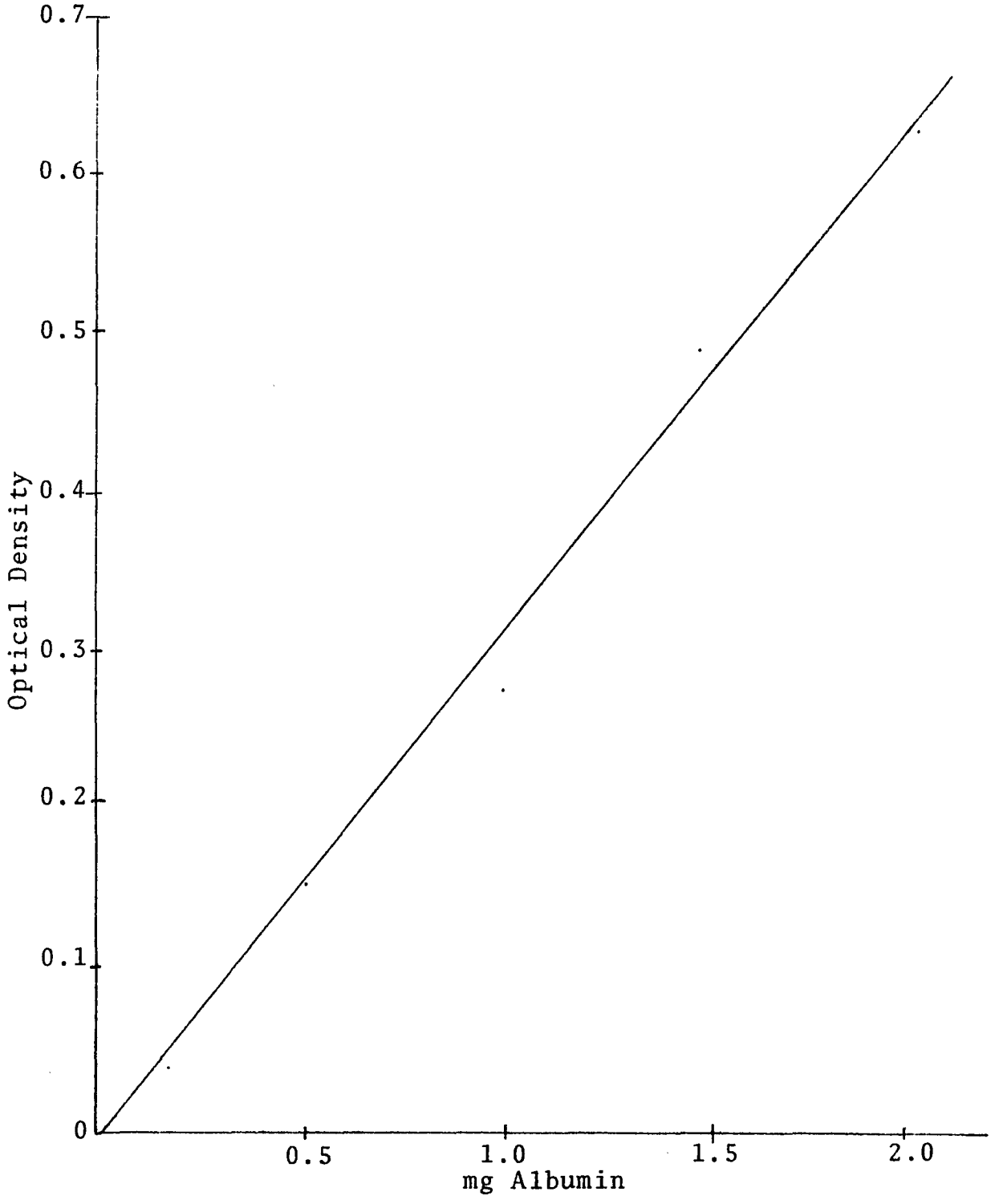

Figure 5. Standard Curve for Bovine Serum Albumin

The tissues of the male reproductive system were stained with Alum Cochineal en toto. They were then dehydrated in graded concentrations of ethanol, cleared in xylene and sectioned at 6μ.

The material for examination with the scanning electron microscope was prepared by suspending sperm in physiological saline and placing 1 drop on a clean glas cover slip or on a piece of muscovite. These preparations were allowed to air dry overnight. The sperm on the glass cover slips were dehydrated in graded concentrations of ethanol and acetone. The sperm were then coated with gold and examined with a Joel Scanning Electron Microscope.

The testes and seminal vesicles filled with sperm were prepared for examination with a transmission electron microscope by dissecting them in physiological saline. Tissues were fixed in 3-6% glutaraldehyde in Millon's Phosphate Buffer at pH 7.3 with the addition of 3% sucrose and 1 mM of calcium chloride. After fixing for 4 hours at 4°C, the tissues were washed overnight in Millon's Phosphate Buffer and postfixed in 1% osmium tetroxide in Millon's Phosphate Buffer for 1 hour at 4°C, rinsed briefly in water, dehydrated in graded concentrations of ethanol and cleared in propylene oxide. The tissue was then embedded in Epon 812 (Luft, 1961) and silver sections cut with a Porter-Blum Ultra-Microtome MT-2. The sections were stained with an aqueous solution of uranyl acetate and counter-stained with

lead hydroxide. The sections were examined with a Hitachi Electron Microscope and with a RCA EMU-3 Electron Microscope.

The sperm for negative staining and for the carbon replicas were suspended in physiological saline. For negative staining a drop of the suspension was placed on a parlodion grid, the sperm allowed to settle and the saline decanted. The sperm was then stained with 1% phosphotungstic acid at pH 7.

A drop of sperm suspension, for carbon replicas, was placed on a parlodion coated grid. The fluid was decanted and the sperm were coated with carbon in an evaporator. The grid was removed from the evaporator and the sperm were digested with 0.15g potassium dichromate in 1.5 ml concentrated sufuric acid. The grids were washed twice with distilled water followed by concentrated hydrochloric acid, then by 3 washings with distilled water. The material was shadowed using a carbon platinum pellet. They were examined with a RCA EMU-3 Electron Microscope.

RESULTS AND DISCUSSION

The spermatozoon of the boll weevil is a filamentous cell, measuring approximately 113.5 μ in length and 0.02 μ in width. Figure 6 is a scanning electron micrograph (SEM) of a complete sperm (arrows). At this magnification it is impossible to discern the head (nucleus and acrosome) and the tail. The sperm appears completely homogeneous and uniform throughout. A portion of the sperm seen in the SEM in Figure 7 appears to possess an undulating membrane. It is unlike the undulating membrane found in urodele amphibians (Barker and Baker, 1970). However, it does contain the axial filament and, therefore, functions as the apparatus that provides motility to boll weevil sperm. The carbon replica seen in Figure 8 shows the axial filament collapsed and it is possible to see the microtubules. An apparently stiff rod with "joints" at regular intervals, a large mitochondrial derivative, can also be seen. Figure 9 is a carbon replica at higher magnification, and the mitochondrial derivative does not demonstrate the "joints" seen in Figure 8. The carbon replica in Figure 10 shows the head of the sperm almost completely composed of nucleus and it is not possible at this magnification to discern the acrosome on the most anterior tip. Immediately at the point at which the nucleus ends, both the mitochondrial derivative

and the axial filament start. Note the other sperm in the picture, where it is possible to distinguish the striations of the large mitochondrial derivative.

The negatively stained sperm in Figure 11 shows 2 mitochondrial derivatives and it is possible to distinguish the striations of the smaller one. The axial filament possesses a wave-like appearance, as if it were undulating.

Figures 12 through 16 are transmission electron micrographs (TEM) showing longitudinal sections of the mature sperm. Figure 12 also shows the wall of the seminal vesicle. Most insect sperm develop in cysts and are bundled together while undergoing spermatogenesis and the various stages of development are precisely synchronized within each cyst (Smith, 1968). When the boll weevil sperm separate from the bundles and are stored in the seminal vesicle they appear to retain this orientation with each other, i.e., one field will have almost all longitudinal sections or mostly cross sections. The acrosome is found on the anterior tip of the nucleus, as is seen in Figures 12, 13 and 14, and appears to be a double structure, i.e., it possesses an outer and inner layer. In Figures 14 and 15 the outer layer appears denser than the inner layer.

The acrosome of the cockroach (Periplaneta americana) appears to be layered in electron micrographs also, and Eddleman et al. (1970) suggested that the layering, i.e., differences in density and texture, is due to chemical differences. Shay and Biesele (1968) found that there are 2

distinct areas apparent within the acrosome of the cave cricket (Ceuthophilus secretus)--a dense inner and less dense outer region. They assumed the inner dense area to be the perforatorium as it is structurally distinct from the surrounding area. Although Hughes and Davey (1969) have shown that the acrosome of the cockroach (Periplaneta) sperm undergoes a change after being stored in the spermatheca of the female, it has not been shown whether the sperm of insects undergo an acrosomal reaction of the type seen in echinoderms or that of mammalian sperm. Neither has it been shown that the acrosome region possesses any proteolytic activity.

It is impossible to distinguish an acrosomal membrane in these TEM's. The nucleus is very darkly stained and appears to have a definite organization or substructure. These figures of longitudinal sections show a number of tangential sections of the axial filament, an indication that the axial filament has a wave-like or undulating motion. Rarely are sections, cross or longitudinal, of the axial filament found on the opposite side of the sperm. This would indicate that the axial filament is found on one side of the sperm and does not spiral around as reported in the flea (Spilopsyllus caniculi) (Smith, 1968). Figure 16 shows a longitudinal section of the axial filament in wave-like movement. It is flanked by dense fibers. Phillips (1970) reports that although young spermatids of insects

contain 2 centrioles, the mature insect sperm possess no centrioles as they disappear during spermiogenesis. No centrioles have been seen during the course of this investigation of the ultrastructure of the boll weevil sperm. It would then seem apparent that in insects sperm centrioles are not necessary for the initiation of cleavage.

There is observed in the sperm of nearly all insects a structure known as the centriolar adjunct (Cantacuzene, 1970). Werner (1965) describes a pericentriolar structure in the sperm of the tiger beetle (Cicindela campestris) and and Cantacuzene (1970) considers this somewhat homologous to the centriolar adjunct. According to Werner (1966) the sperm of the ground beetle (Carabus catenulatus) have a centriolar adjunct.

Neither a centriolar adjunct nor any pericentriolar structure were observed in any of the electron micrographs of boll weevil sperm.

Some of the sperm seen in Figures 13, 14, 15 and 16 contain 2 mitochondrial derivatives (nebenkern), one much larger than the other. This confirms the studies of Phillips (1970) who stated that 2 mitochondrial derivatives are usual for insect sperm, one larger than the other; however, these mitochondrial derivatives can be of equal size or there may exist only one such derivative as found in many Trichoptera. The nebenkern of the boll weevil appear to extend from the base of the nucleus throughout

its entire length. In all the shallow longitudinal sections of the mitochondrial derivatives, they exhibit a segmented appearance. Andre (1962) described the mitochondrial derivatives (paracrystalline component) as periodically striated when viewed in longitudinal section.

Figures 18 through 21 are TEM's of cross sections of the mature boll weevil sperm. They readily exhibit the large mitochondrial derivative as a large circular structure with a herringbone pattern, having the appearance of a rather stiff rod. The two structures just above the large nebenkern have been referred to (Gassner, personal communication) as fibrillar material of unknown origin and function, but possibly related to the mitochondrial derivative. Figures 21 and 22 show these two structures in longitudinal section (arrows) and they possess the segmentation or striations that the large mitochondrial derivative also shows in longitudinal section. Some of the figures in longitudinal section show both the large mitochondrial derivative and the small mitochondrial derivative. In cross section these 2 structures do not possess the same type of paracrystalline material, i.e., the smaller 2 do not have the same herringbone pattern as the larger one. However, according to Phillips (1970), mitochondrial derivatives in sperm of some insect species contain 2 morphologically distinct types of paracrystalline material. However, no insect sperm has been reported to have more than

2 mitochondrial derivatives. Gassner (personal communication) referred to the small, dense horizontal V-shaped structure indicated by arrows in Figure 20 as the small mitochondrial derivative. He indicated that it was often masked by the fibrillar material. In none of these sections does it ever appear separate, and it always appears confluent with one or the other of the fibrillar structures. It does not consistently appear on the same fibrillar structure. More work is necessary to clearly identify these structures.

The dense fibers flanking the axial filament are identified by Gassner (personal communication) as fibrillar material of unknown origin and function also. Shay and Biesele (1968) described supporting elements of the undulating membrane located between the mitochondrial derivatives and the axial filament in the cave cricket. These structures, although flanking the axial filament in the boll weevil sperm, may well function also as supporting elements of the undulating membrane.

The axial filament consists of a 9 + 9 + 2 arrangement of microtubules. As seen in all the cross sections there is an outer ring of 9 accessory fibers. Cameron (1965) described this outer ring as arising as outgrowths from the outermost side of the B subfibers in Tenebrio sperm. The outer ring in both the boll weevil and Tenebrio consists of single units and the inner ring consists of

doublets. Baccetti (1970) surveyed the sperm structure in the phylum Arthropoda and concluded that the basic 9 + 2 arrangement of the flagellum is not efficient for internal fertilization without an aqueous medium. Nine outer accessory fibers occur in most of the Endopterygota for which studies have been reported.

Figures of the cross sections do not indicate how the axial filament functions as a motile apparatus. In Figure 22 are sections of the sperm which are not true cross sections and demonstrate that the sperm is capable of flattening out and the axial filament functioning as an undulating membrane.

All of the cross sections show a membrane, the plasmalemma, surrounding the whole sperm. All of the excess cytoplasm has been sloughed off during speriogenesis and none remains in the mature sperm.

It was impossible to study the ultrastructure of the sperm from the seminal vesicle of the Louisiana or Mexico hybrid. These hybrid males do not produce enough sperm to store in the seminal vesicle, therefore sections of the testis were studied instead of seminal vesicle sperm. The testis of the boll weevil, thurberia weevil, and their hybrids were studied at the light and electron microscopy level and the hybrids reveal a disrupted pattern of spermatogenesis.

Figure 23 is a light micrograph (LM) of the testis

of the boll weevil showing a very orderly pattern of spermatogenesis with the sperm arranged in bundles as indicated by the groups of very darkly staining heads. Figure 24 is a LM of the thurberia weevil testis and its orderly pattern of spermatogenesis with bundled sperm. At this level of magnification, it is impossible to distinguish the various stages of spermatogenesis.

Figure 25 is a LM of the testis of the Mexico hybrid and even at this level of magnification one observes disruption of spermatogenesis. There are no bundles of mature sperm. The normal sperm that may be present seemingly have trouble moving into the seminal vesicle. The same situation is apparent in the Louisiana hybrid testis, as shown in Figure 26. When compared with normal testis, the hybrid testis appears to be chaotic. This is even more evident in Figure 27, which is a portion of the Louisiana hybrid testis at higher magnification.

When the testes are studied with an electron microscope, the chaotic condition is even more startling. Figures 28 and 29 are TEM's of sections of the boll weevil and Figure 30 is of the thurberia weevil. The first impression is that orderliness prevails in both. Each cell is approximately at the same stage of development. Some are undergoing mitosis and are therefore out of synchrony. One axial filament forming is seen in each cell. Figure 29 shows that the 9 outer fibers are dense when first formed

and become less dense or hollow only later in development (see Figures 18, 19 and 20). Numerous microtubules are seen surrounding the other structures within the cell. The dense particles around the periphery of the cells are glycogen particles. More of these are apparent in young spermatids than in the older spermatids, e.g., the spermatids in Figure 30 are younger than those in Figure 29.

Figures 31 through 36 are TEM's of the Louisiana hybrid testis, Figures 31 and 32 are apparently cross section and the remainder are longitudinal or saggital sections. The cross sections show more inclusions in the hybrid than occur in normal testes. Most contain 2 axial filaments instead of the normal 1. The size of the cell differs, i.e., there is no uniformity or orderliness. The situation is even more exaggerated in Figure 32 than 31. The longitudinal sections also illustrate this chaotic condition. In Figures 35 and 36 the structure located at the base of the nucleus on either side of the axial filament may be construed to be the centriolar adjunct. At the tip of the nucleus in one cell in Figure 34, an acrosomal vesicle may be seen. The chromatin of the spermatids has not undergone condensation to any great extent.

In the Mexico hybrid, the condition is almost impossible to describe. Figures 37 through 41 are TEM's of the Mexico hybrid testes and the pictures represent complete chaos. Fragmentation appears to be going on, especially in

Figure 37. Frequently the components appear to be doubled. It is obvious that the process of spermatogenesis has broken down and instead of the orderliness that characterizes the normal testes, disorder and chaos characterize the hybrid testes.

The diploid chromosome number of the boll weevil is 22 (Lue et al., 1973). The thurberia weevil also has a diploid number of 22. Squash preparations of the testes show several chromosomes assuming a ring configuration at metaphase I in the boll weevil. Squash preparations of the thurberia weevil testes do not show as many chromosomes assuming this ring configuration. This observation may indicate that not all the chromosomes of the thurberia weevil and the boll weevil are homologous. Darlington (1929) suggested that homologues must be held together by chiasmata in order to pass to opposite poles at the first meiotic anaphase. If this normal reduction division does not occur, abnormal secondary gametocytes will be produced which, in turn, would lead to abnormal production of sperm. It is generally accepted that chiasmata are associated with crossing over. Crossing over occurs during the time the chromosomes are in the synaptonemal complexes; a synaptonemal complex forms from homologous chromosomes (King, 1972). Since the boll weevil and thurberia weevil chromosomes do not appear to be homologous, a true synaptonemal complex may form. Perhaps there is no crossing over and

no chiasmata formed and normal gametogenesis is disrupted. In insects crossing-over usually occurs in one sex only. In Drosophila it occurs in the female only (Morgan, 1912, 1914). Since the F_1 females of boll weevil x thurberia weevil crosses and the reciprocal are fertile (Newsom, unpublished results), perhaps crossing-over occurs only in the male boll weevil. It appears that in hybrid spermatogenesis, cytoplasmic division may not occur since several of the inclusions appear duplicated. However, Lindsley and Grell (1969) have shown in Drosophila melanogaster that the presence of the chromosome complement in the spermatid nucleus is not necessary for its normal differentiation into a functional spermatozoon. The full chromosome complement must be present in the primary spermatocyte nucleus. In Drosophila there are factors located on the Y chromosome which seem to control the coordination of the various synthetic and morphogenetic processes in spermatids leading to the formation of functional sperm without necessarily contributing structural information on the molecular level. Therefore, the XO male Drosophila is sterile. The sex-determining mechanism of most of the curculionid weevils species (Takenouchi, 1965) show the males to be the heterogametic sex through their possession of an Xy, usually Xyp, chromosomes. It is possible, perhaps, that the male boll weevil needs the full chromosome complement for fertility. Hess and Meyer (1968), however, have suggested that in

Drosophila the nutritive cell associated with each developing sperm bundle plays a decisive role in directing development. The sperm in the hybrid testes are not in bundles indicating the absence of nurse cells and this suggests the possibility that their presence is necessary for the normal development of boll weevil sperm. At this time it is impossible to determine why aberrant spermatogenesis occurs in these hybrids.

In the boll weevil after the sperm have undergone spermiogenesis, they separate from the bundles and move to the seminal vesicle where they are stored until mating occurs. The physical stimulation of the male aedeagus seems to be the trigger for sperm to leave the seminal vesicle. Shortly after leaving the seminal vesicle, the sperm become mixed with the secretion from the accessory glands. The secretion stimulates the sperm since they move more vigorously in a suspension of the accessory gland material in physiological saline. In contrast, when normal sperm are placed in a suspension containing accessory glands from hybrid males the sperm cease moving almost immediately. This reaction seems to indicate that the accessory gland secretions differ in the normal boll weevil male and the hybrid male.

Kahn and Musgrave (1969) have shown that the secretion of the "prostate gland" of Sitophilus activates the sperm. Davis (1964) has also shown in Cimex lectularius

that the seminal fluid is necessary for fertilization and without it the sperm do not migrate. The accessory glands of the boll weevil are obviously of 2 kinds; one is hyaline and larger in diameter, the other is opaque, thin and longer. When subjected to protein analysis by the microburet method, the optical density of the hyaline glands is 0.02 and of the opaque is 0.365. This indicates a great difference between the secretion of each of the glands.

The sperm continue to move down the vas deferens assisted by rhythmic contractions of the vas deferens. When they reach the "ring" just before entering the chitinous aedeagus they stop, accumulate and form a mass (Figure 42). It appears as if the sperm are preparing for spermatophore formation, although one never forms. Davey (1960) cited several criteria as evidence for the formation of a spermatophore by males: 1) presence of accessory glands in male, 2) a bursa copulatrix as an internal receptacle for the spermatophore in females, and 3) the possession of a rather shortened penis. Of the 3 criteria, 2 are found in the boll weevil, i.e., accessory glands in the male and bursa copulatrix in the female. The male has developed a somewhat elongate penis. It is suggested here that the ancestor of the boll weevil produced a spermatophore and the present day boll weevil still possesses most of the structures necessary for the transfer of sperm in a

spermatophore, although it transfers free sperm.

After the sperm have accumulated in the vas deferens during copulation, they are transferred to the female and deposited in the bursa copulatrix (copulatory pouch). The sperm then move to the storage organ of the female, the spermatheca, until required for fertilization of eggs as they pass down the median oviduct. The mechanism involved in the transfer of sperm from the bursa to the spermatheca has perplexed investigators for some time. Hinton (1964) and Davey (1965) have thoroughly reviewed the work in this area. Davey (1958) has concluded that the transfer in Rhodnius prolixus is a result of rhythmic contractions set up in the oviduct by the opaque accessory gland secretion of the male acting through a peripheral nervous system. Jones and Wheeler (1965) concluded that in Aedes aegyptii the behavior of the sperm alone is not capable of explaining normal spermathecal filling and the role of the female is not clear. Kahn and Musgrave (1969) speculate that in Sitophilus the sperm are sucked into the spermatheca, which, by means of its musculature and wrinkled wall could act as a pump. Ruttner et al. (1971) concluded that in the queen bee the transfer of sperm from the oviducts to the spermatheca is a complex process involving the queen's musculature, the fluids of her spermatheca and its gland, as well as the spontaneous movement of the sperm.

There is evidence that sperm will swim upstream,

i.e., against the current (Walton, 1952; Roberts, 1970). Weidner (1934) stated that in Bombyx copulation triggers the activity of the spermathecal gland so that a copious flow of secretion from the receptaculum into the ductus results and migration of the sperm is the result of a positive chemotactic response to the secretion.

In the boll weevil copulation triggers a flow of material from the spermatheca down the spermathecal duct and sperm can be seen swimming against the current toward the spermatheca. The flow of material in the spermathecal duct is never seen in virgin females. Contractions of the common oviduct are seen in any and all females. In mated females they do not have any effect on the movement of sperm to the spermatheca.

The material stored in the virgin female's spermatheca, secreted by the spermathecal gland, does activate and attract the sperm. When a spermatheca from a virgin female devoid of sperm is broken open in a sperm suspension the immediate reaction is that the sperm very rapidly aggregate around the break and activity is tremendously increased. This reaction on the part of the sperm is comparable to the reaction of sperm of Nereis and Arbacia to jelly coat (fertilizin) of the eggs (Lillie, 1919). Ermert (1970) has shown that the secretion of the boll weevil spermathecal gland is a neutral mucopolysaccharide. Runnström (1952) concluded that the jelly coat or fertilizin is an acidic

mucopolysaccharide. He also found that when sperm are suspended in jelly solutions their fertilizing capacity is considerably prolonged, despite the increased motility of the sperm. Koeniger (1970) also concluded that the secretion of the spermathecal gland of the queen bee contains a factor which is essential for the locomotion and fertilizing capacity of the sperm. Therefore, it is suggested that a similar role could be ascribed to the spermathecal gland of the female boll weevil.

The results of this study show that when a female boll weevil is mated with a fertile male and 4 days later mated with a sterile male, the eggs produced are preferentially penetrated by sperm from the second mating. About 80% of the sperm from the first mating appear to have been displaced by sperm from the second mating, i.e., after the second mating 80% of the eggs do not hatch. These results are shown in Table I. Parker (1970) found this also is true in multiple mating of Scatophaga stercoraria. Lefevre and Jansson (1962) also found in Drosophila melanogaster that sperm from a second mating will displace those from the first. Gilliland and Davich (1966) investigated the effects of alternate mating of the boll weevil using sterile males and found that the last mating prior to oviposition was most influential on subsequent egg viability. Likewise, Bartlett et al. (1968) used a genetic marker, pearl-colored eyes, to investigate multiple matings and use

TABLE I

Sperm Displacement Following Multiple Mating

	After mating with fertile male				After mating with sterile male			
♀ #	# laying days	# eggs laid	# eggs hatched	% hatch	# laying days*	# eggs laid	# eggs hatched	% hatch
1	4	42	38	90.5	31	307	96	31.3
2	4	51	47	92.2	50	421	105	24.9
3	4	30	28	93.3	14	178	27	15.2
4	4	25	23	92.0	20	163	50	30.7
5	4	34	32	94.1	38	280	71	25.4
7	4	18	17	94.1	2	27	5	18.5
8	3	8	7	87.5	14	83	12	14.5
9	4	43	35	81.4	15	148	27	18.2
11	4	35	35	100.0	3	4	4	100.0
12	4	67	62	92.5	11	192	17	8.9
13	4	38	36	94.7	48	406	125	30.8
14	1	1	1	100.0	25	181	55	30.4
15	4	51	48	94.1	14	246	53	21.5
Totals:		444	409	92.1		2,766	673	24.3

*The spermatheca of all females contained no sperm after egg-laying ceased.

of sperm in the boll weevil. They found that sperm from the last mating took precedence over sperm from other matings only when at least 24 hours separated the mating. This advantage of sperm from the last mating ranged from about 10% when 1 day separated matings to 90% when 4 days intervened. The data from the present study supports the findings of the previous studies on the boll weevil. Sperm from the second mating takes precedence when a sufficient amount of time elapses between the two matings, i.e., sperm from a second mating displace sperm of the first mating from the spermatheca.

CONCLUSIONS

Boll weevil sperm consists of an acrosome, nucleus, 2 or 3 mitochondrial derivatives and an axial filament with a 9 + 9 + 2 arrangement of microtubules. There is 1 pair, possibly 2, of fibrillar material of unknown origin and function.

The hybrid sterility found in the F_1 males from the cross of thurberiae males and grandis females is the result of aberrant spermatogenesis. This disruption of spermatogenesis may be the result of chromosomal incompatibility or failure of the sperm to bundle properly. It is not possible to state definitively the reasons spermatogenesis is aberrant in these hybrids.

A possible model of sperm transfer in the boll weevil starts with copulation triggering a flow of spermathecal material down the spermathecal duct. This spermathecal material activates and attracts the sperm which have been introduced into the bursa of the female. The sperm then swim upstream into the spermatheca. The spermathecae store the sperm for considerable periods of time and the secretion of the spermathecal gland preserves the fertilizing capacity of the sperm. When a second mating follows the first within 4 days, the spermathecal flow is again triggered and approximately 80% of the sperm from the

first mating are carried out of the spermatheca by the flow of material from the spermatheca. The displaced sperm remain in the median oviduct and vagina and are probably expelled to the outside when the male withdraws from the female or when the eggs are laid.

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Figure 6. SEM of boll weevil sperm.
2,600 X.

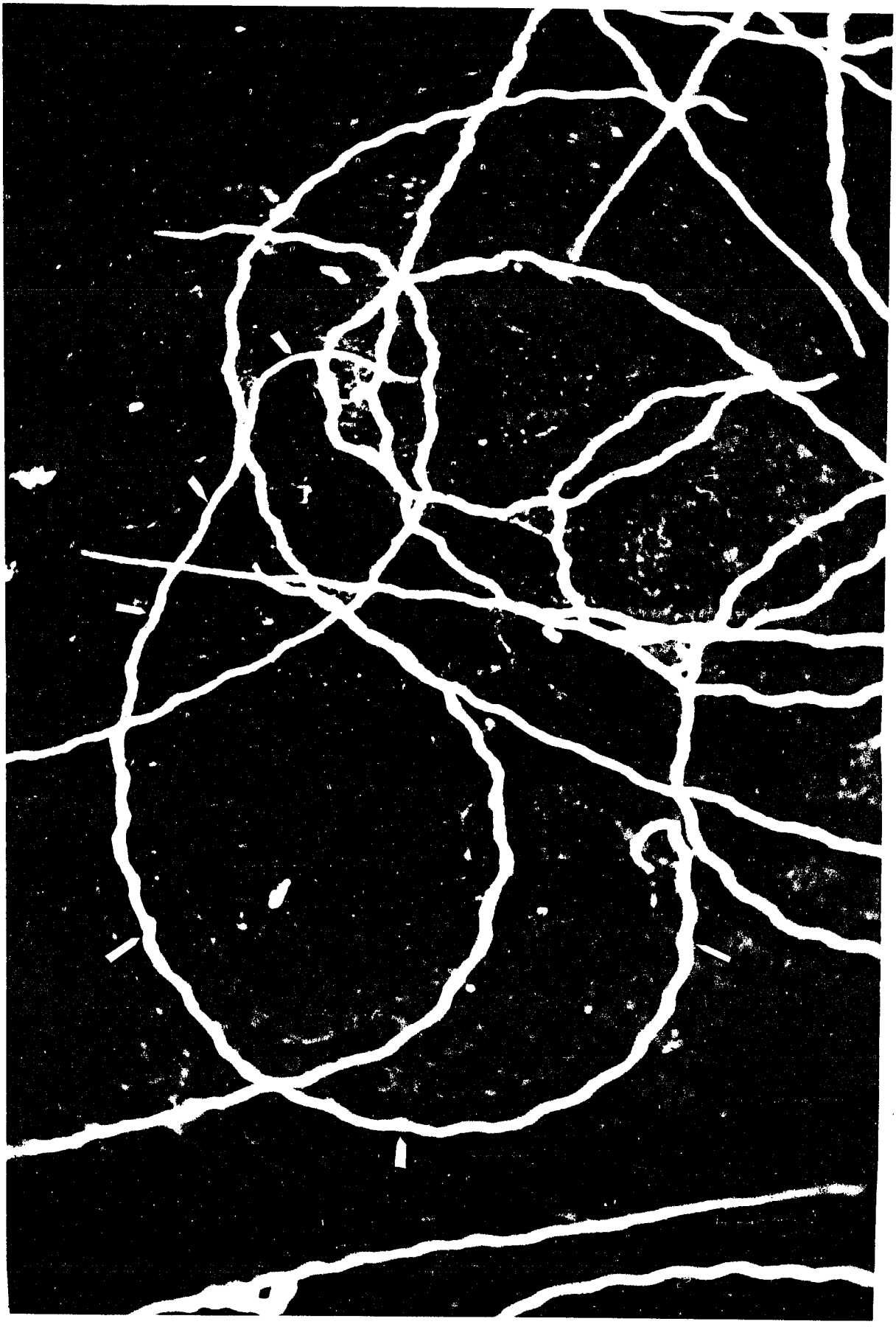

Figure 7. SEM of a portion of boll weevil sperm. 10,400 X.

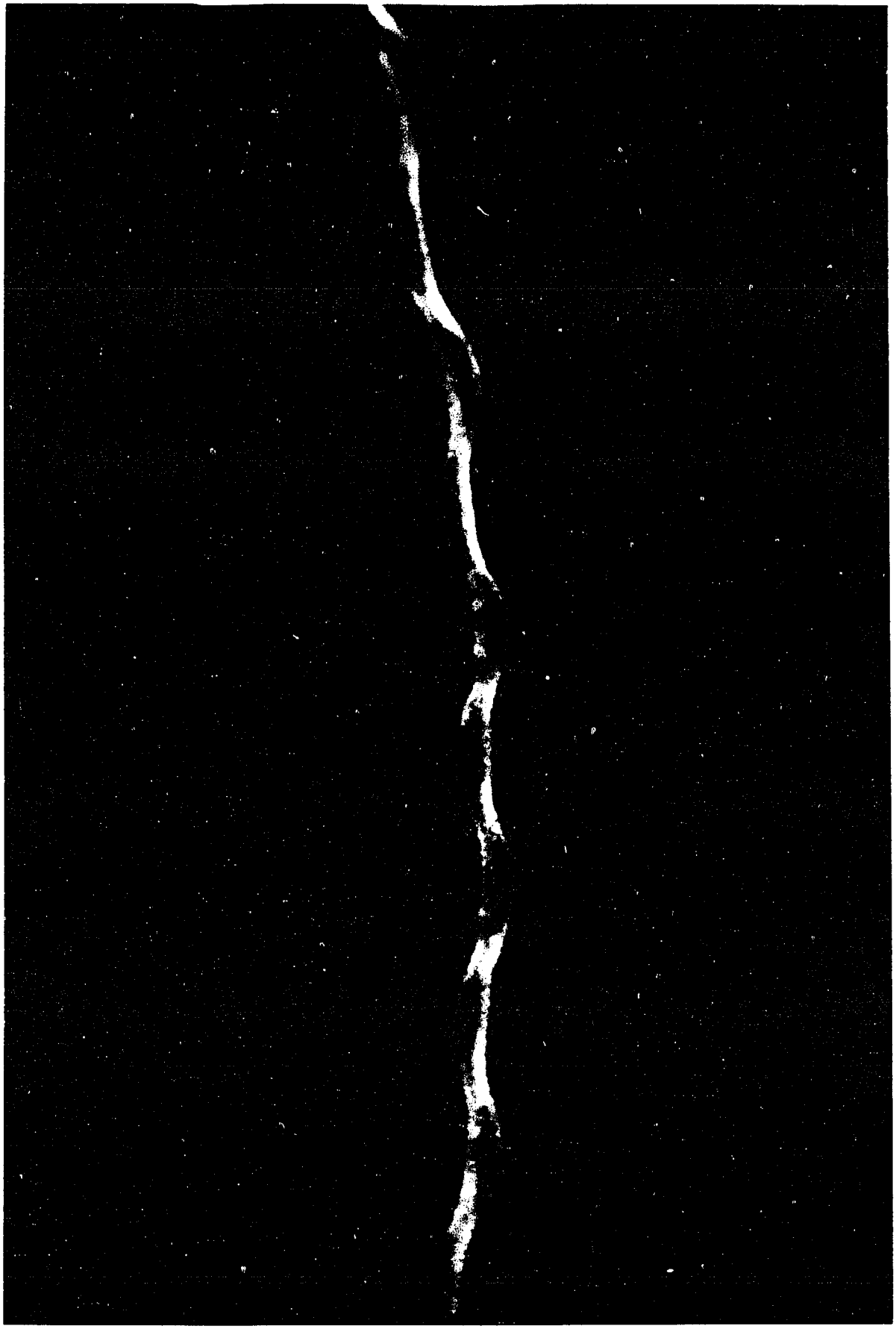

Figure 8. Carbon replica of a portion of a boll weevil sperm.

16,000 X.

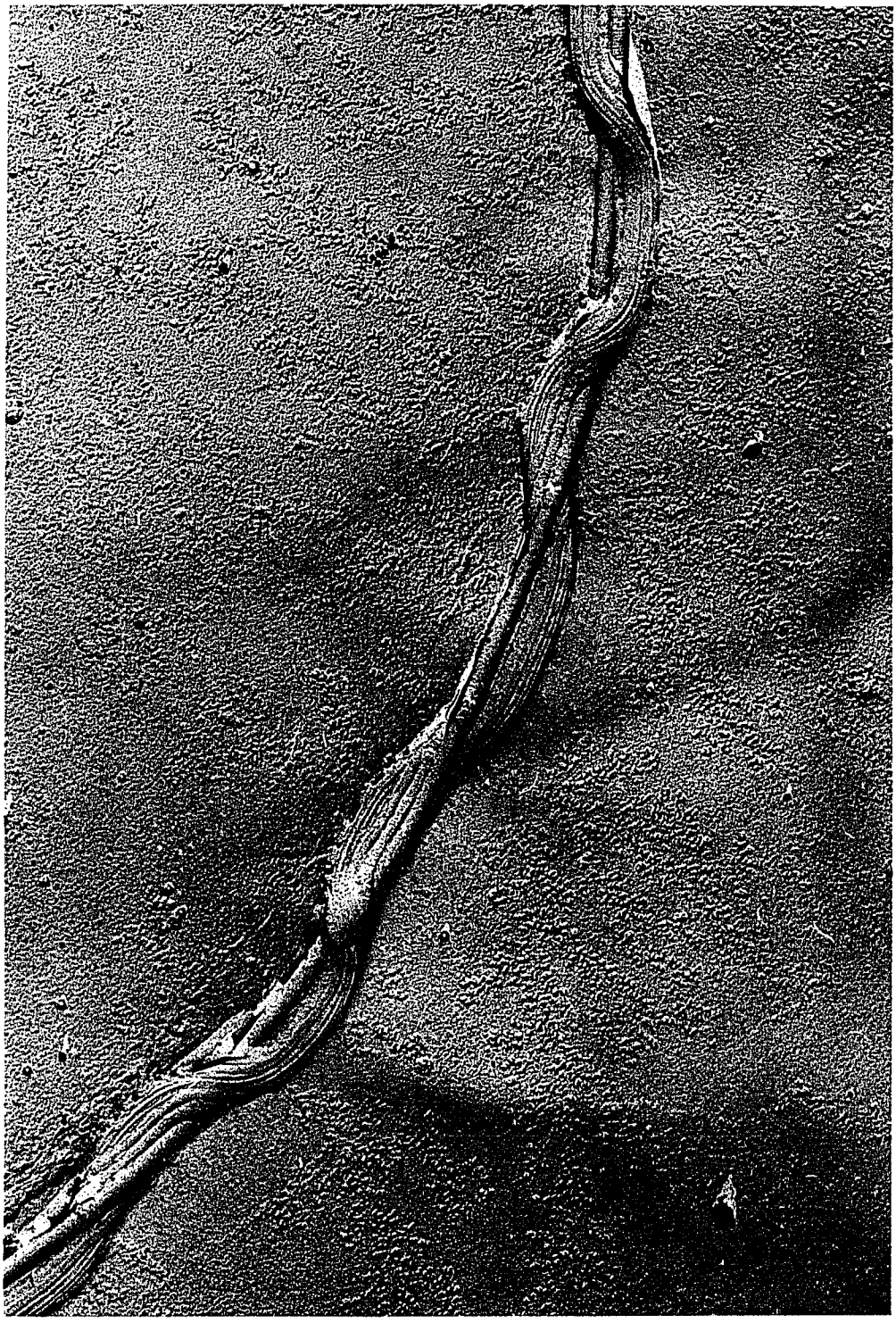

Figure 9. Carbon replica of portions of several boll weevil sperm.

23,000 X.

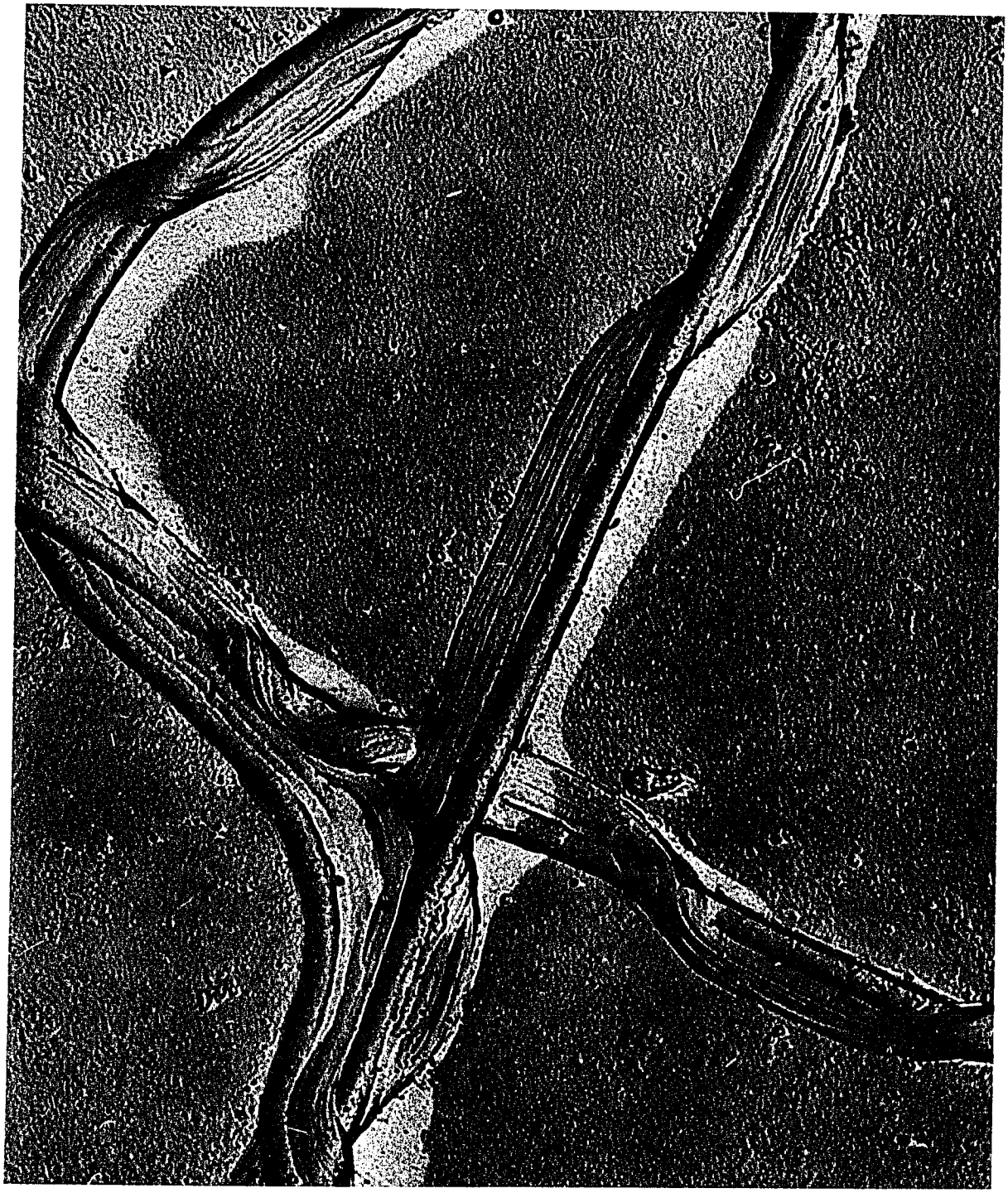

Figure 10. Carbon replica of anterior portion of boll weevil sperm.

23,000 X.

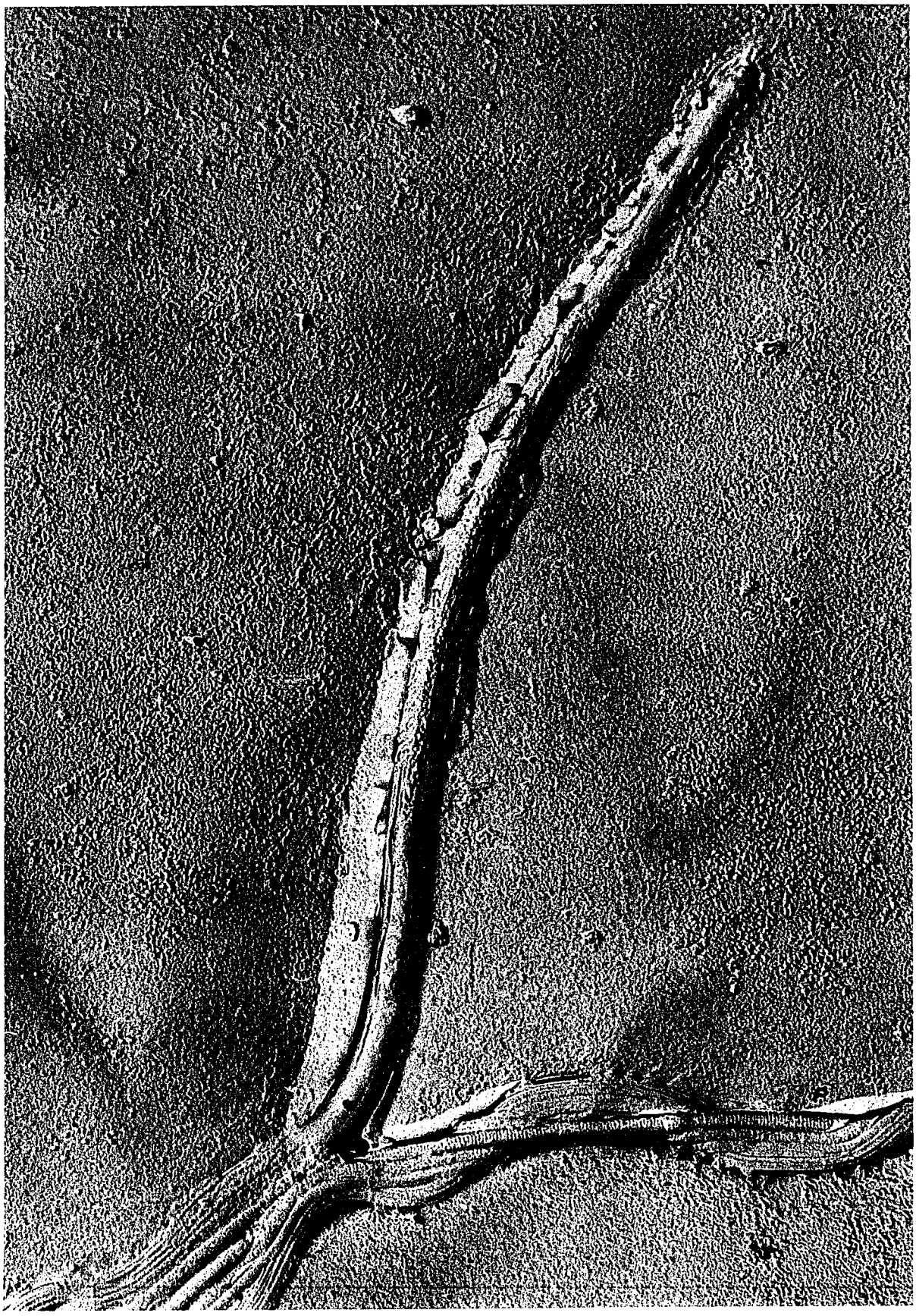

Figure 11. Portion of a negative stained sperm. 23,000 X.

Figure 12. TEM showing seminal vesicle sperm, showing wall of seminal vesicle.

7,800 X.

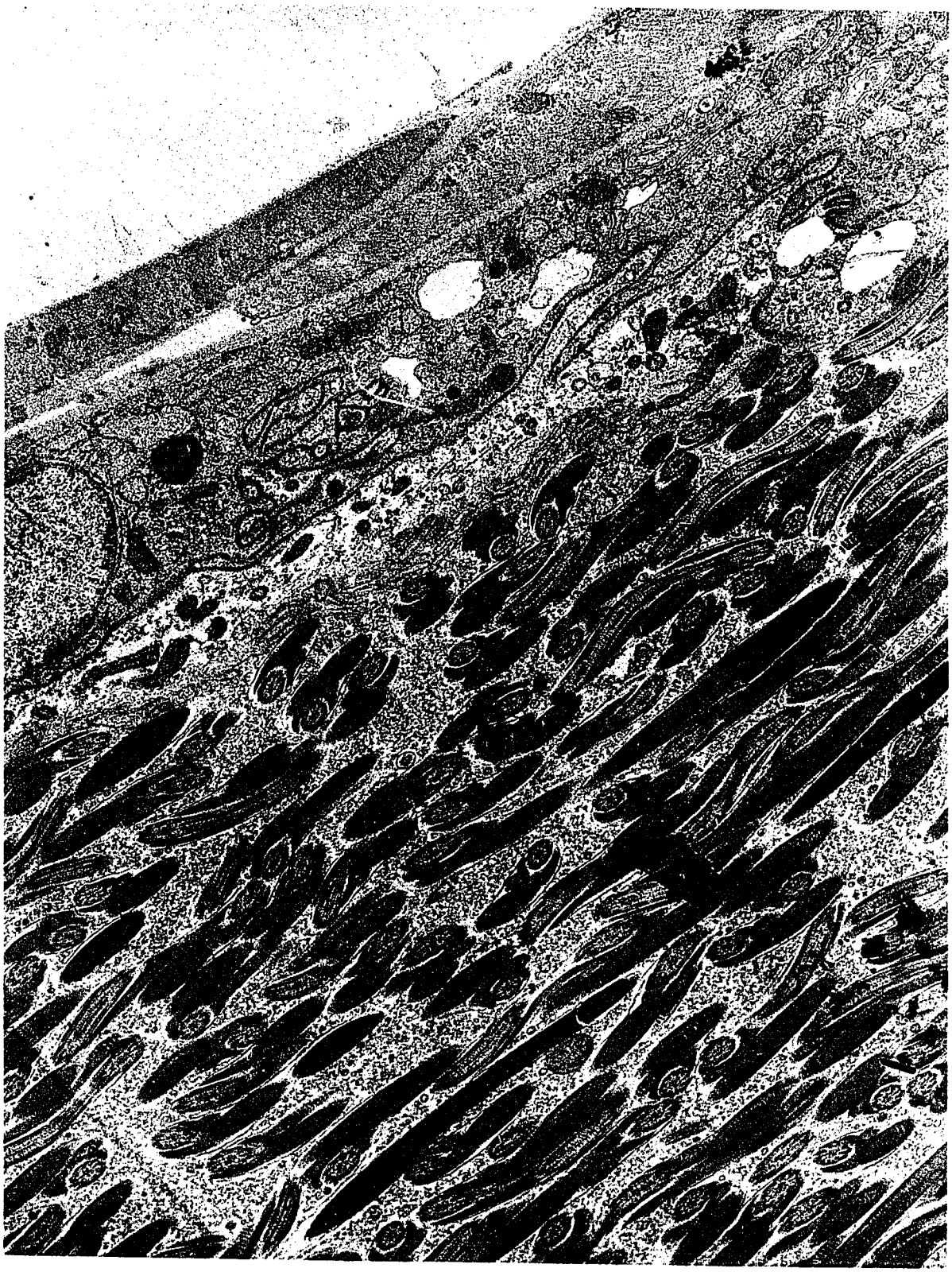

Figure 13. TEM, longitudinal sections of boll weevil sperm.

11,000 X.

Figure 14. TEM, longitudinal section of boll weevil sperm.

30,800 X.

Figure 15. TEM, longitudinal section of boll weevil sperm.

30,800 X.

Figure 16. TEM, longitudinal section of boll weevil sperm.

37,000 X.

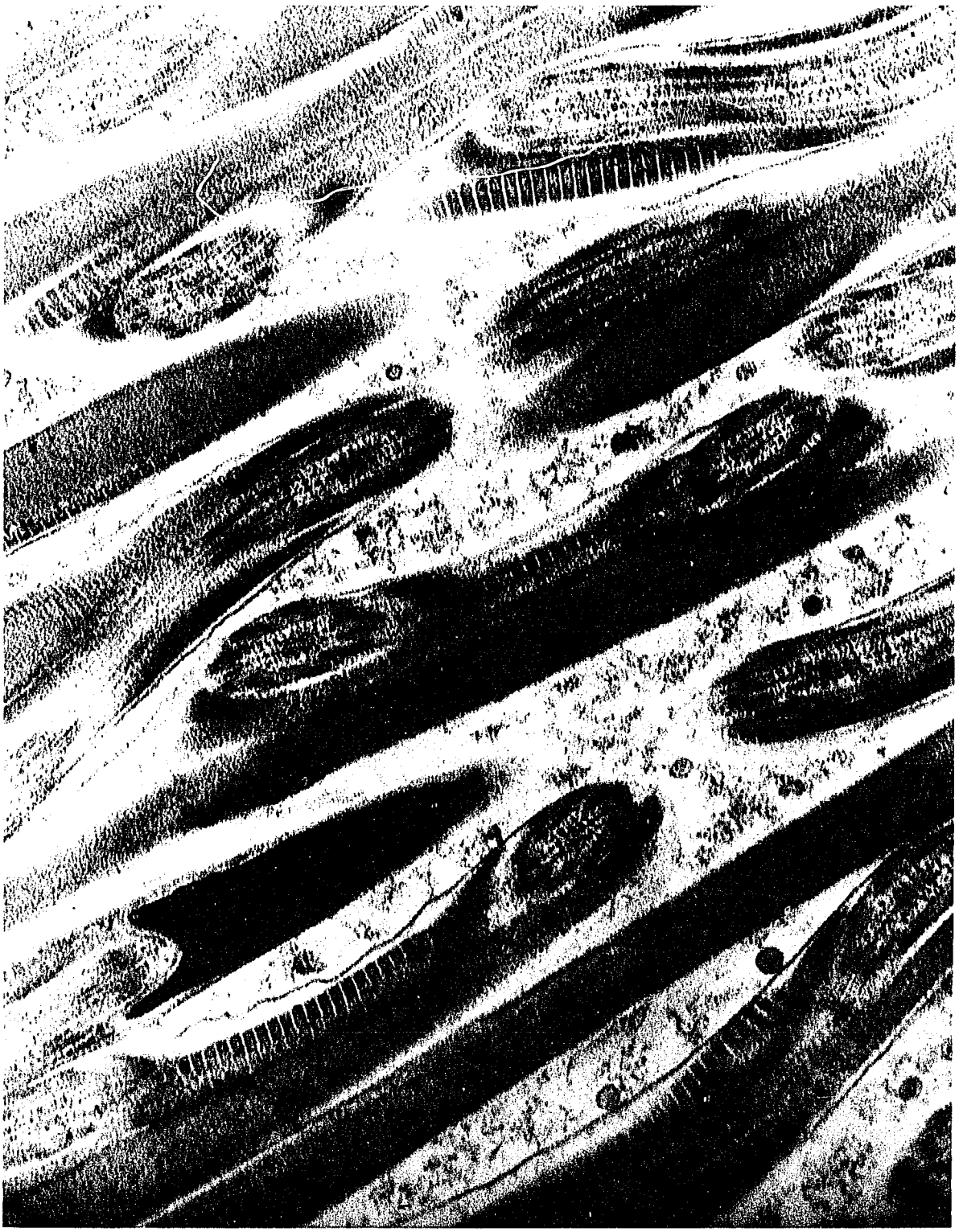

Figure 17. TEM, longitudinal section of boll weevil sperm.

33,000 X.

Figure 18. TEM cross sections of boll weevil sperm. 97,000 X.

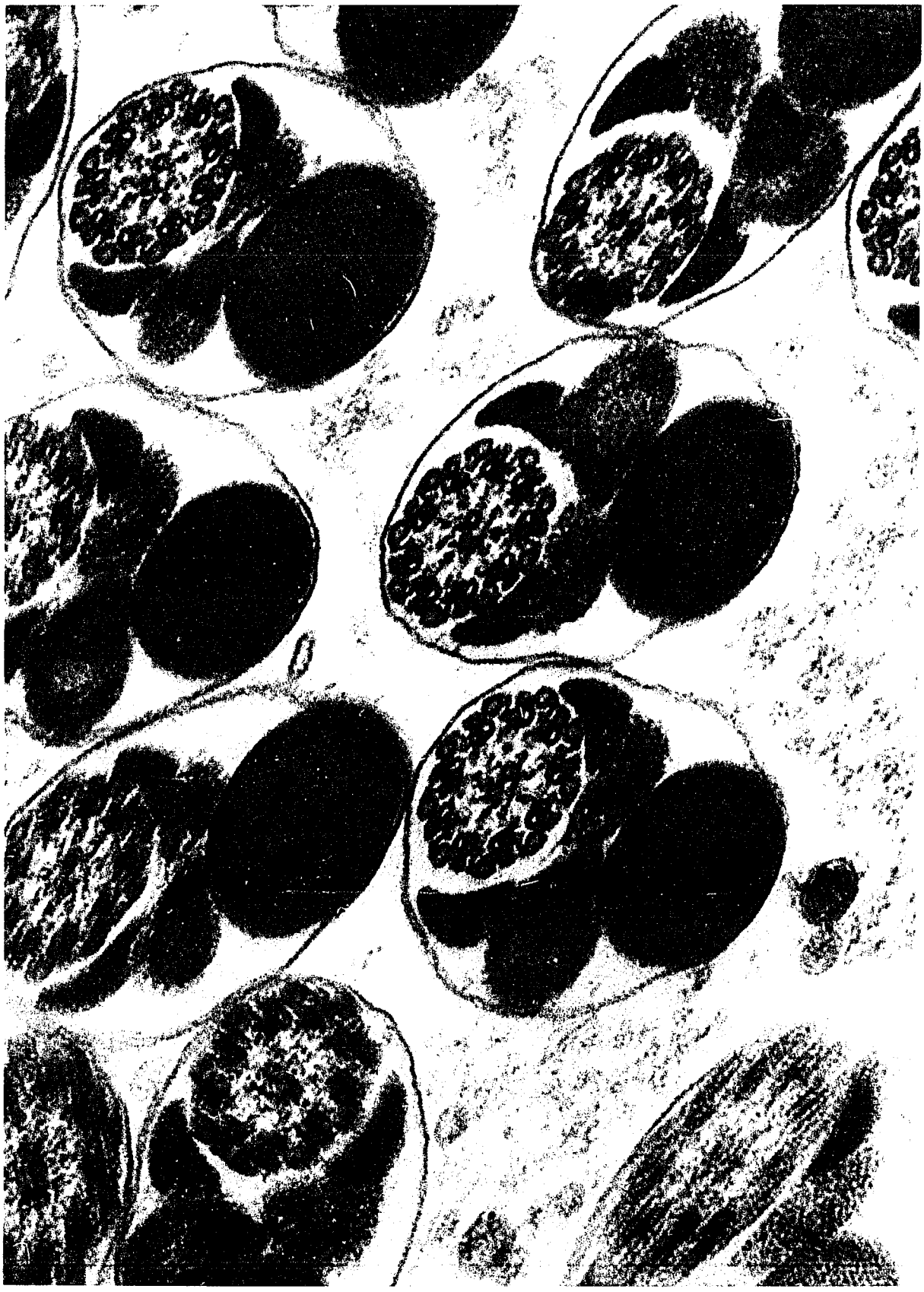

Figure 19. TEM cross sections of boll weevil sperm. 46,000 X.

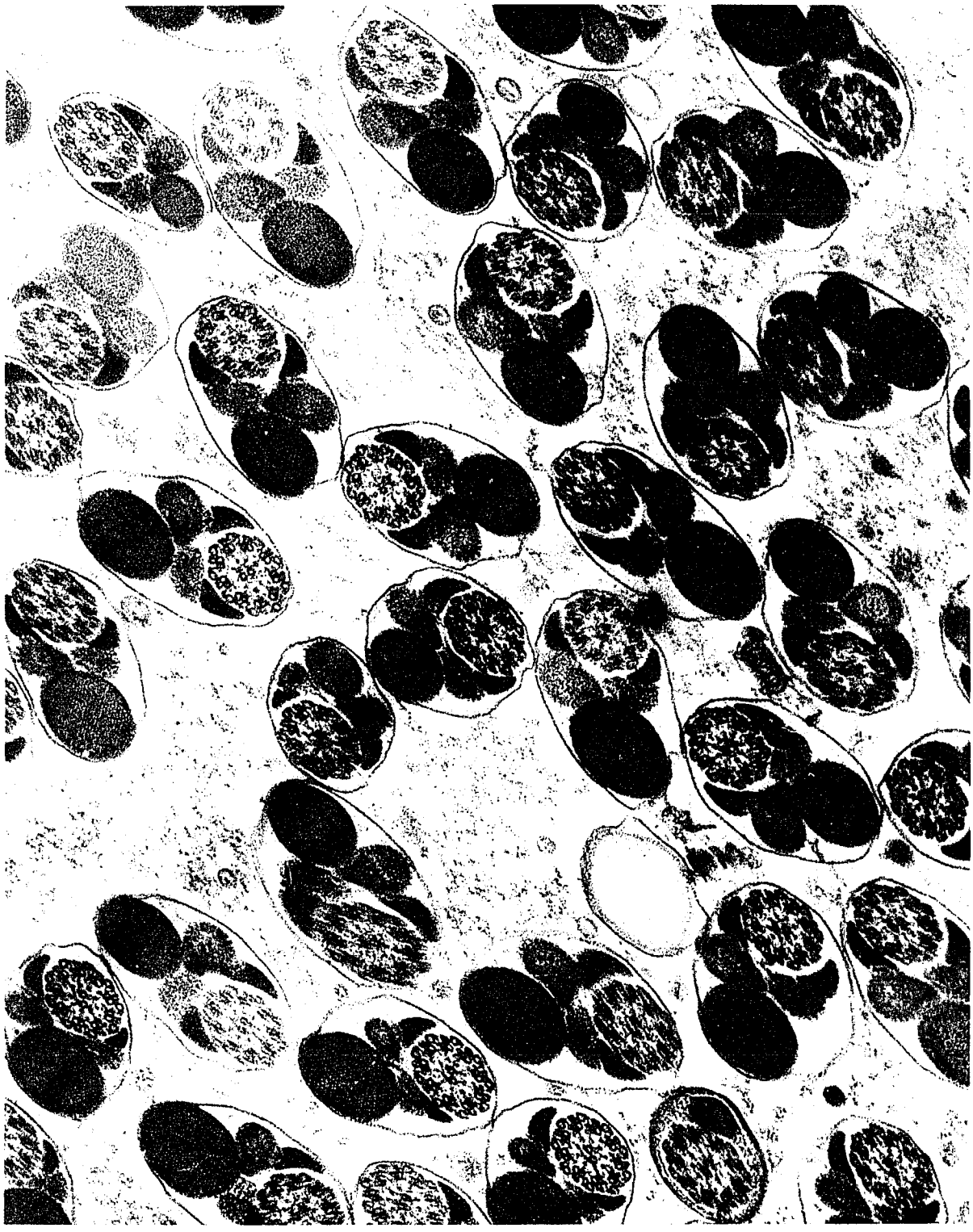

Figure 20. TEM cross sections of boll weevil sperm. 61,000 X.

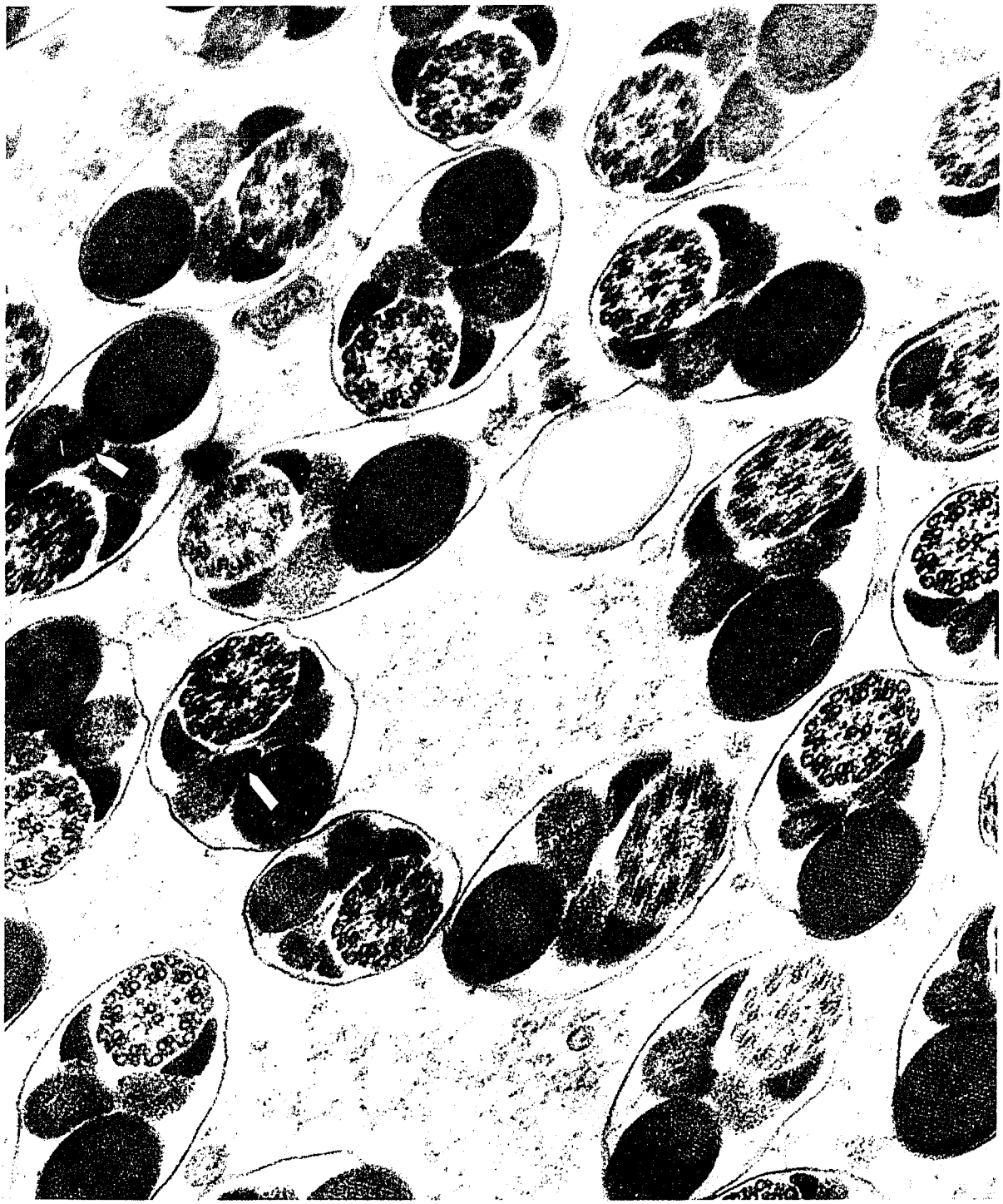

Figure 21. TEM cross sections of boll weevil sperm. 64,000 X.

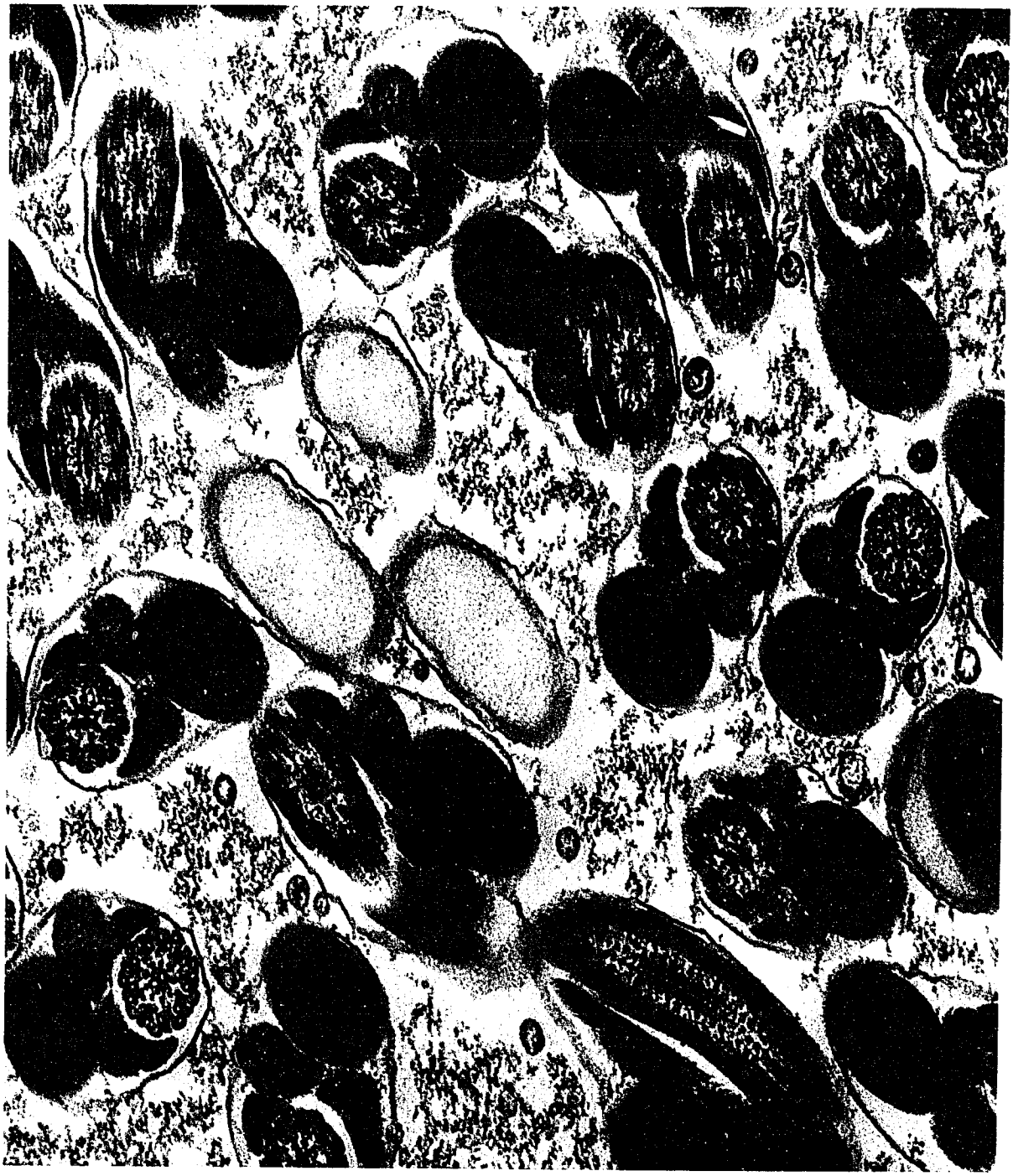

Figure 22. TEM cross and longitudinal sections of boll weevil sperm.

44,000 X.

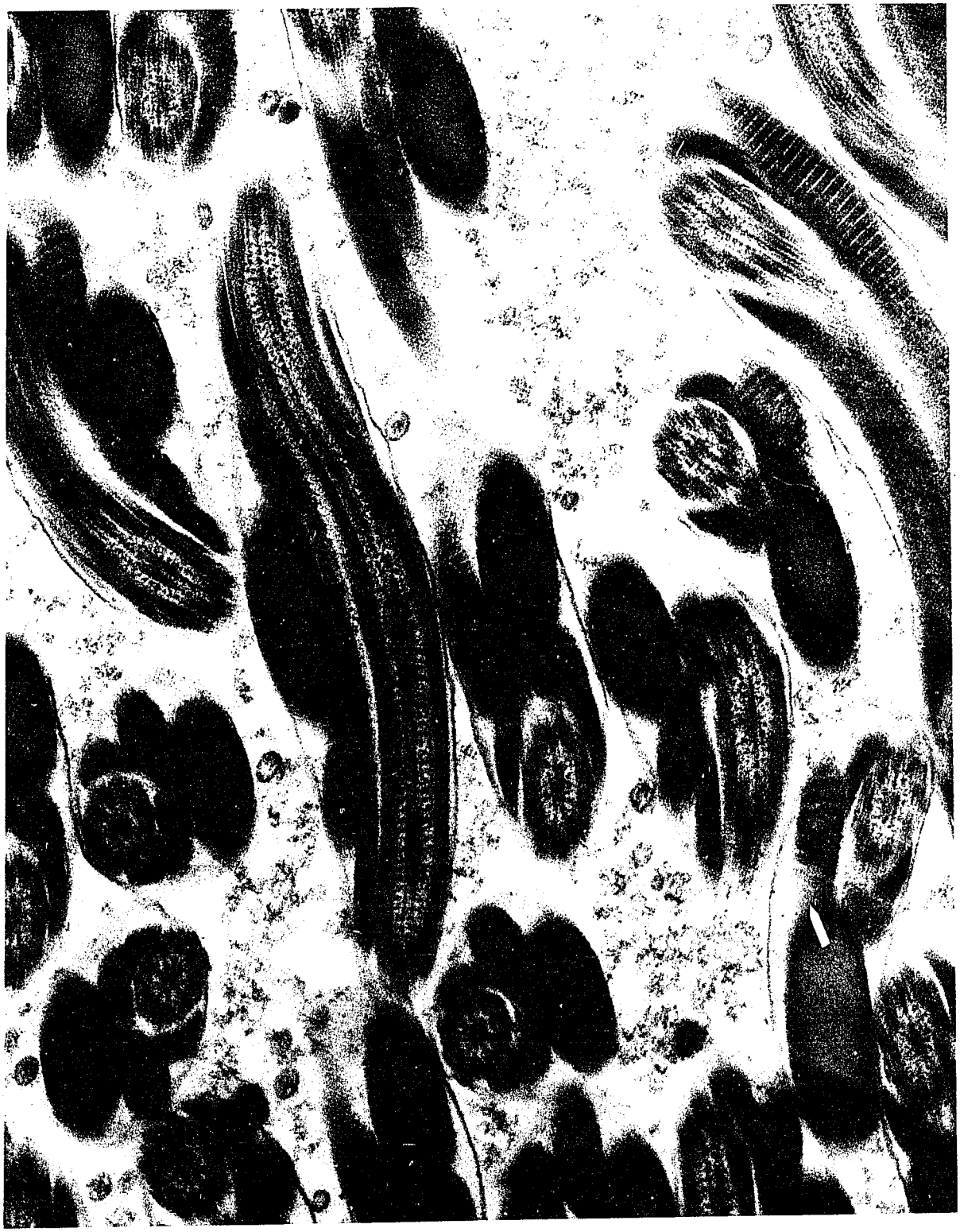

Figure 23. LM, normal boll weevil testis section. 300 X.

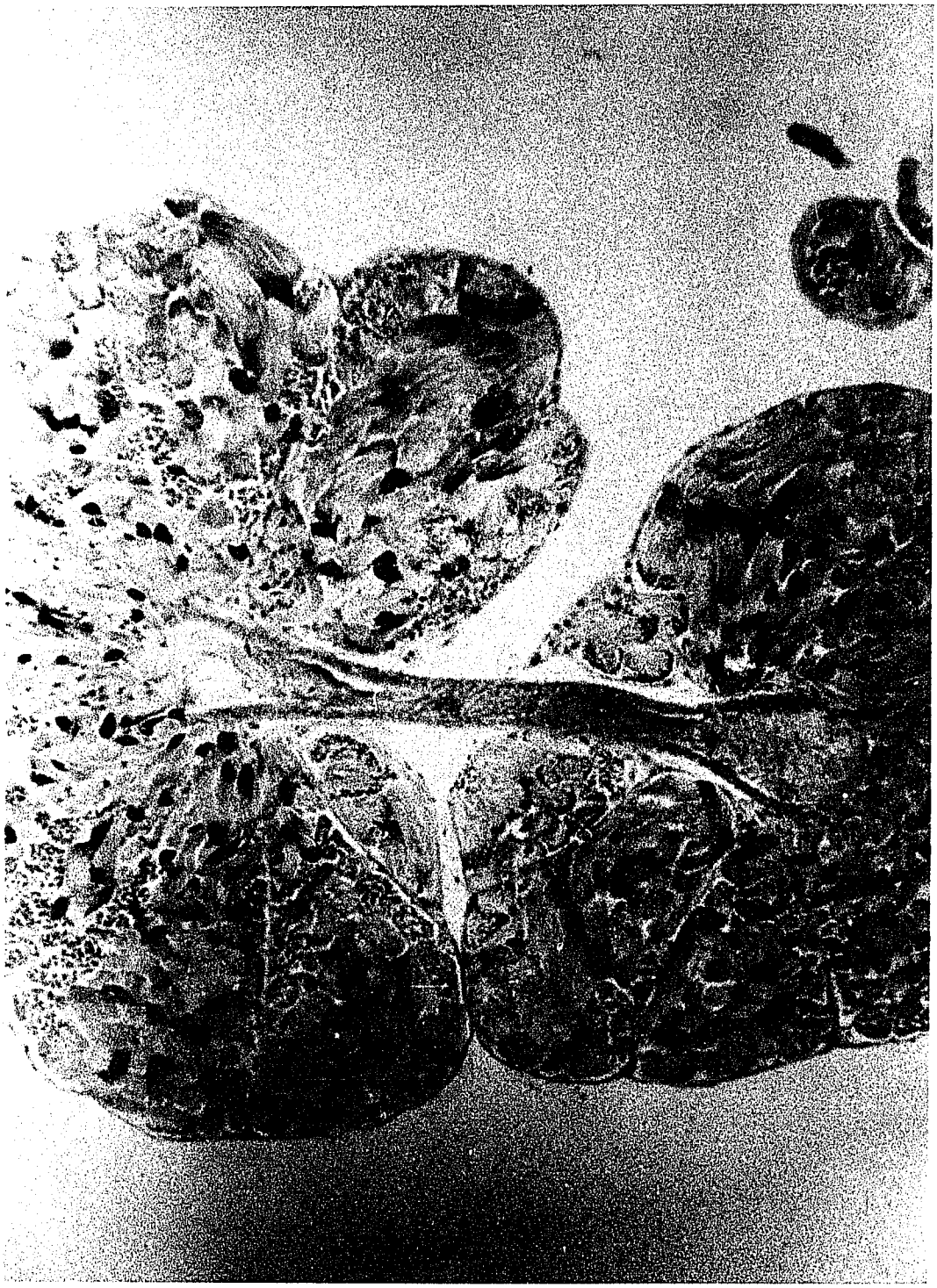

Figure 24. LM, section of thurberia testis. 560 X.

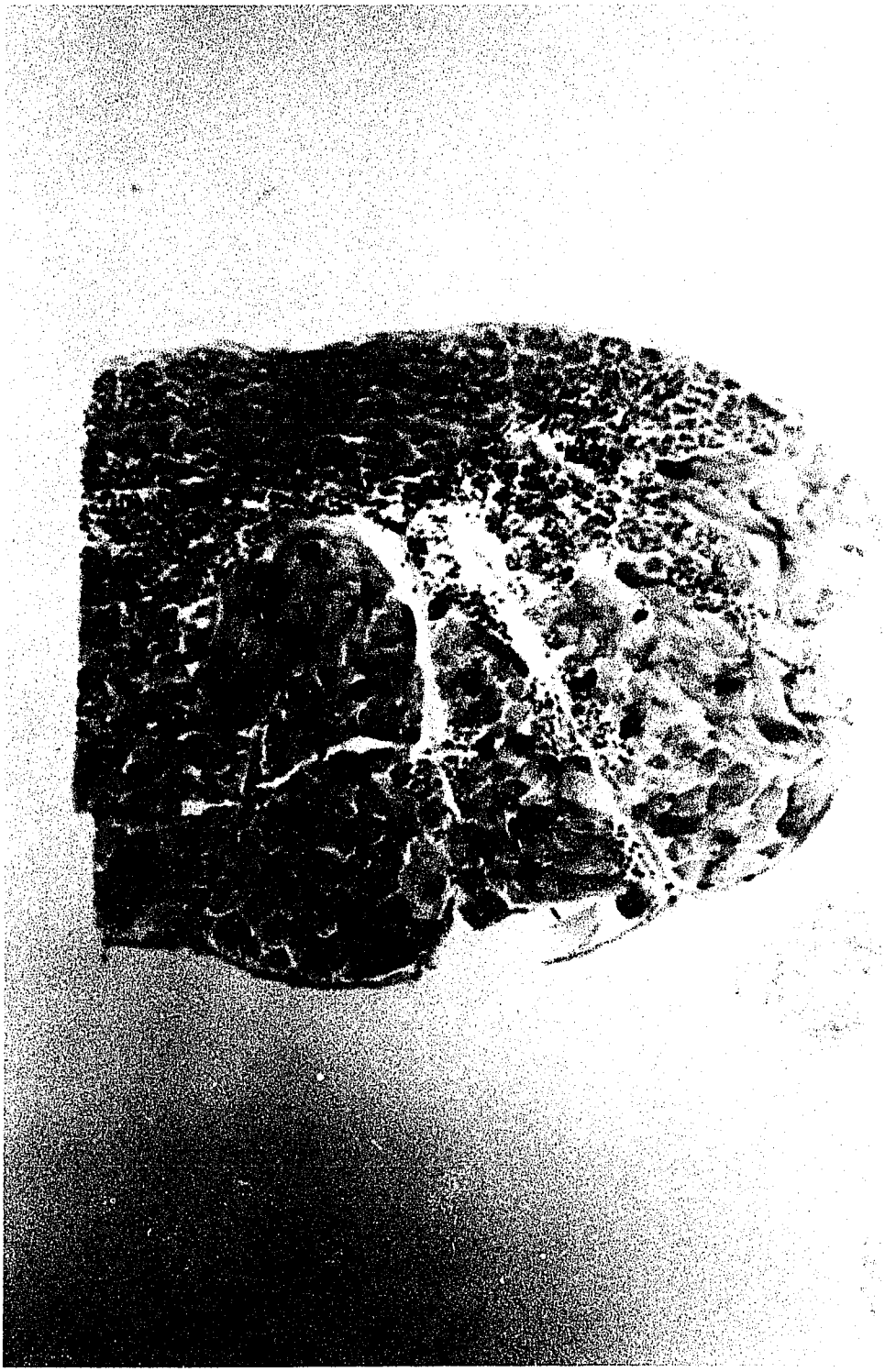

Figure 25. LM, section of Louisiana hybrid testis. 300 X.

Figure 26. LM, section Mexico hybrid testis. 300 X.

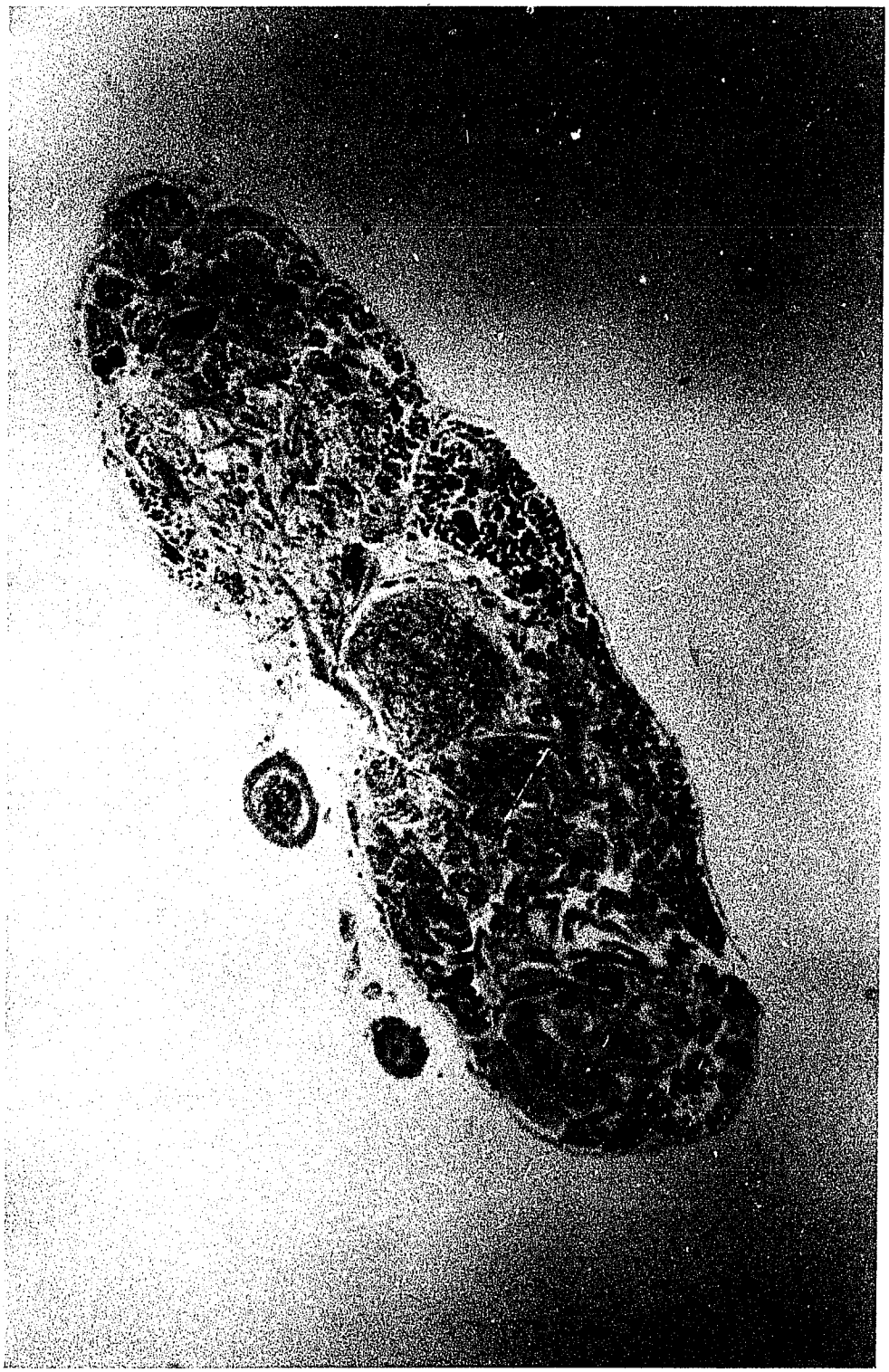

Figure 27. LM, section Mexico hybrid testis. 760 X.

Figure 28. TEM cross section of spermatids in boll weevil testis.

23,400 X.

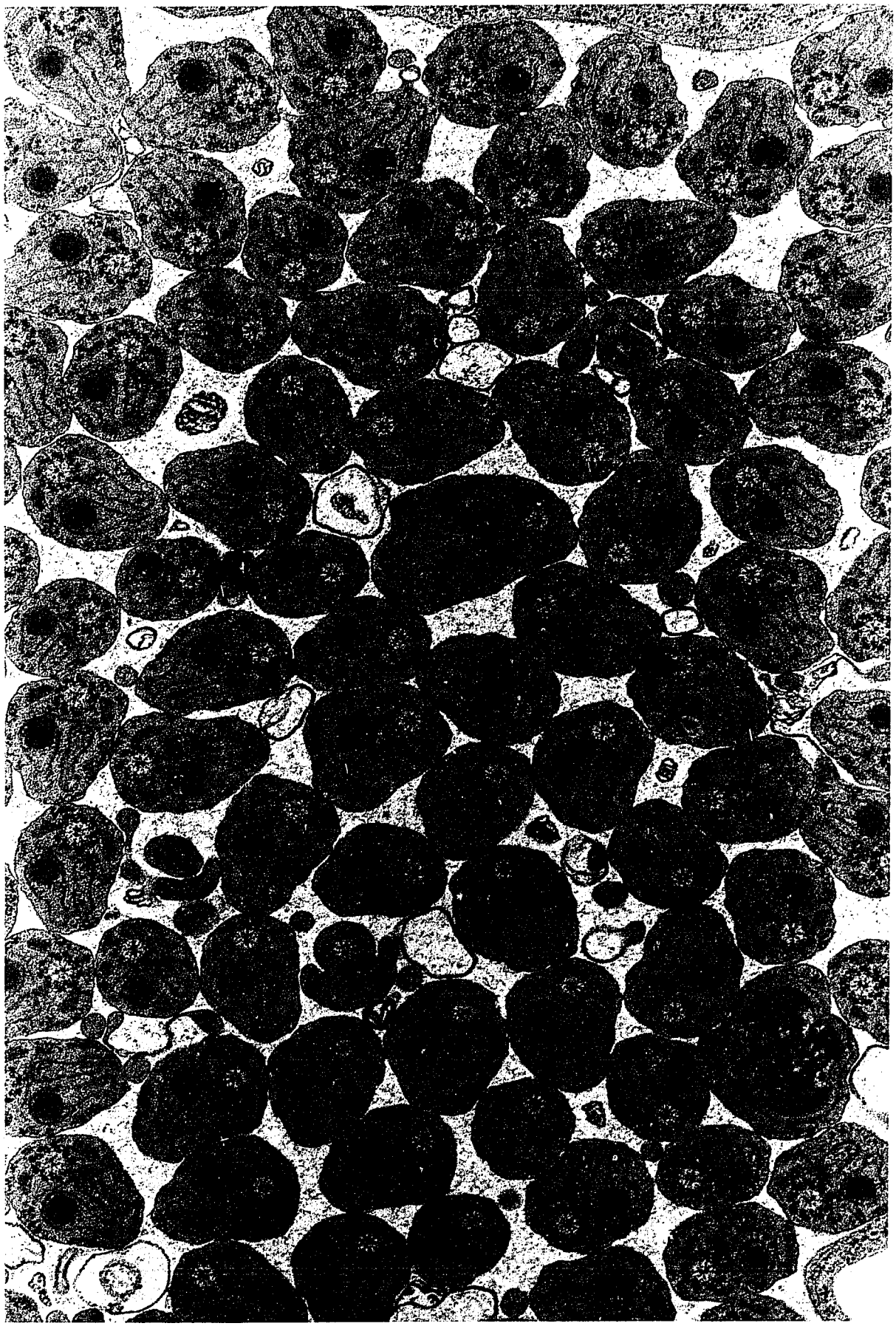

Figure 29. TEM cross section of spermatids in boll weevil testis.

68,000 X.

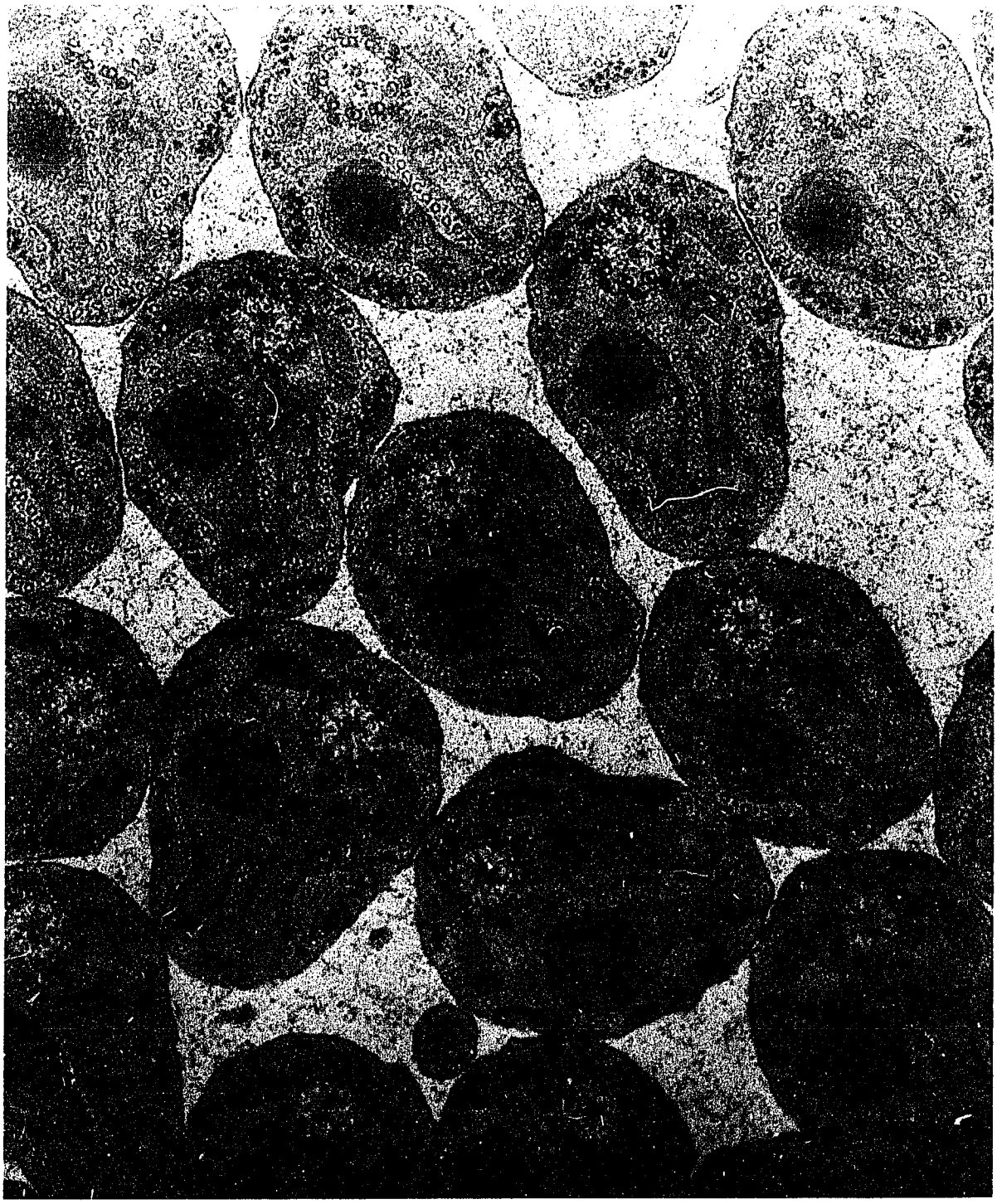

Figure 30. TEM cross sections of spermatids of thurberia testis.

20,000 X.

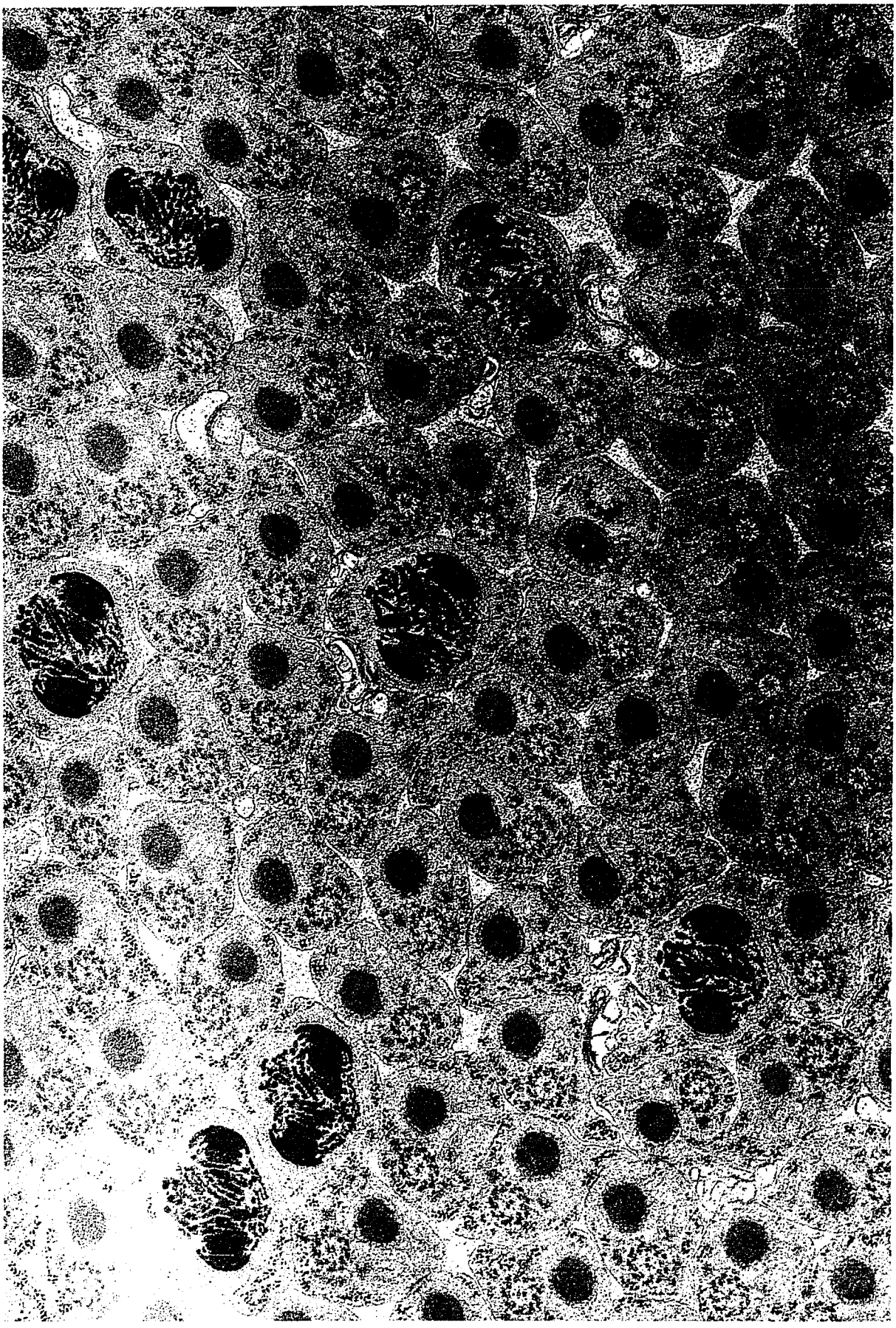

Figure 31. TEM of section of Louisiana hybrid testis.

43,000 X.

Figure 32. TEM section of Louisiana hybrid testis. 22,000 X.

Figure 33. TEM section of Louisiana hybrid testis. 22,000 X.

Figure 34. TEM section of Louisiana hybrid testis. 15,000 X.

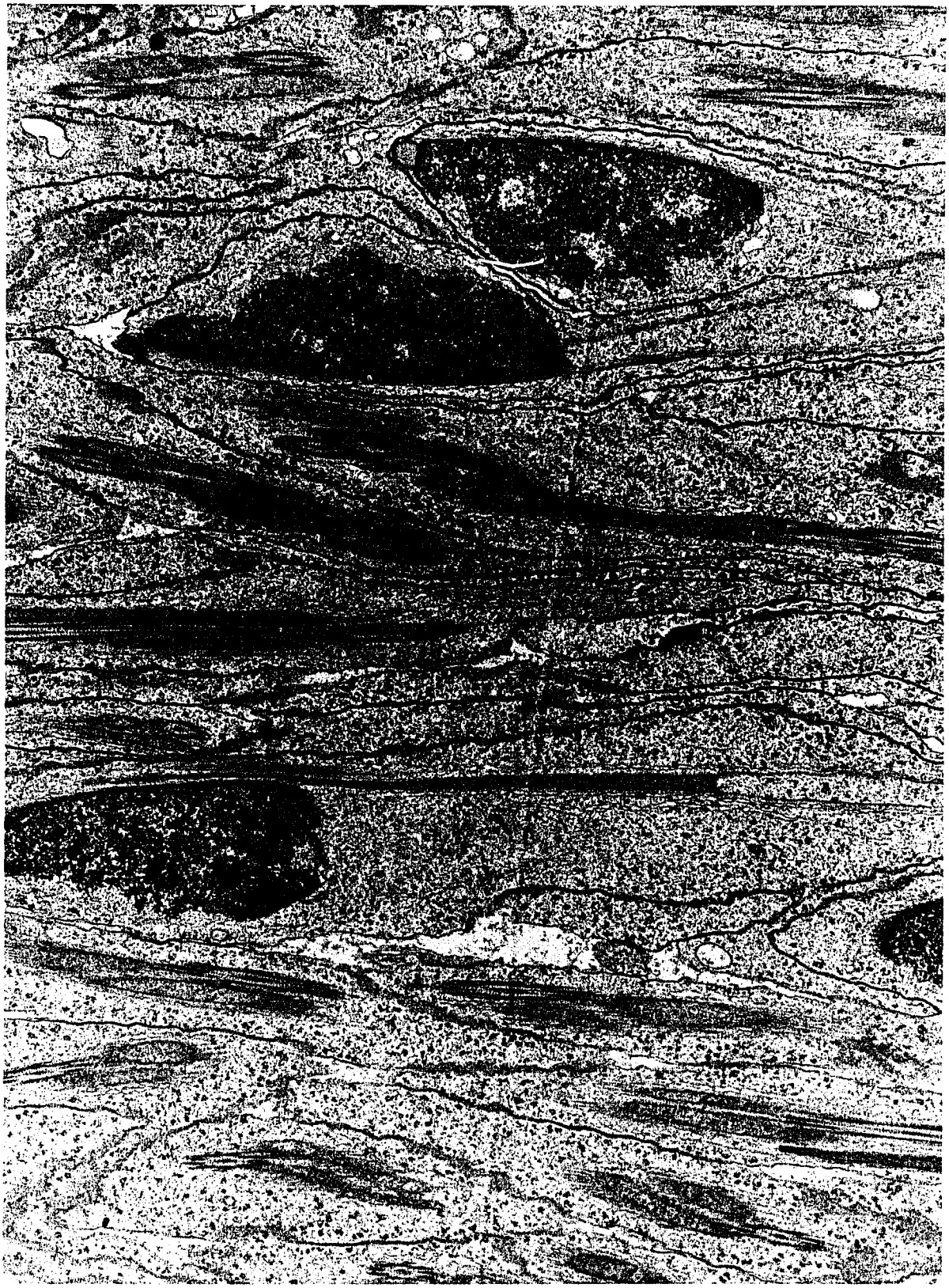

Figure 35. TEM section of Louisiana hybrid testis 12,000 X.

Figure 36. TEM section of Louisiana hybrid testis. 22,000 X.

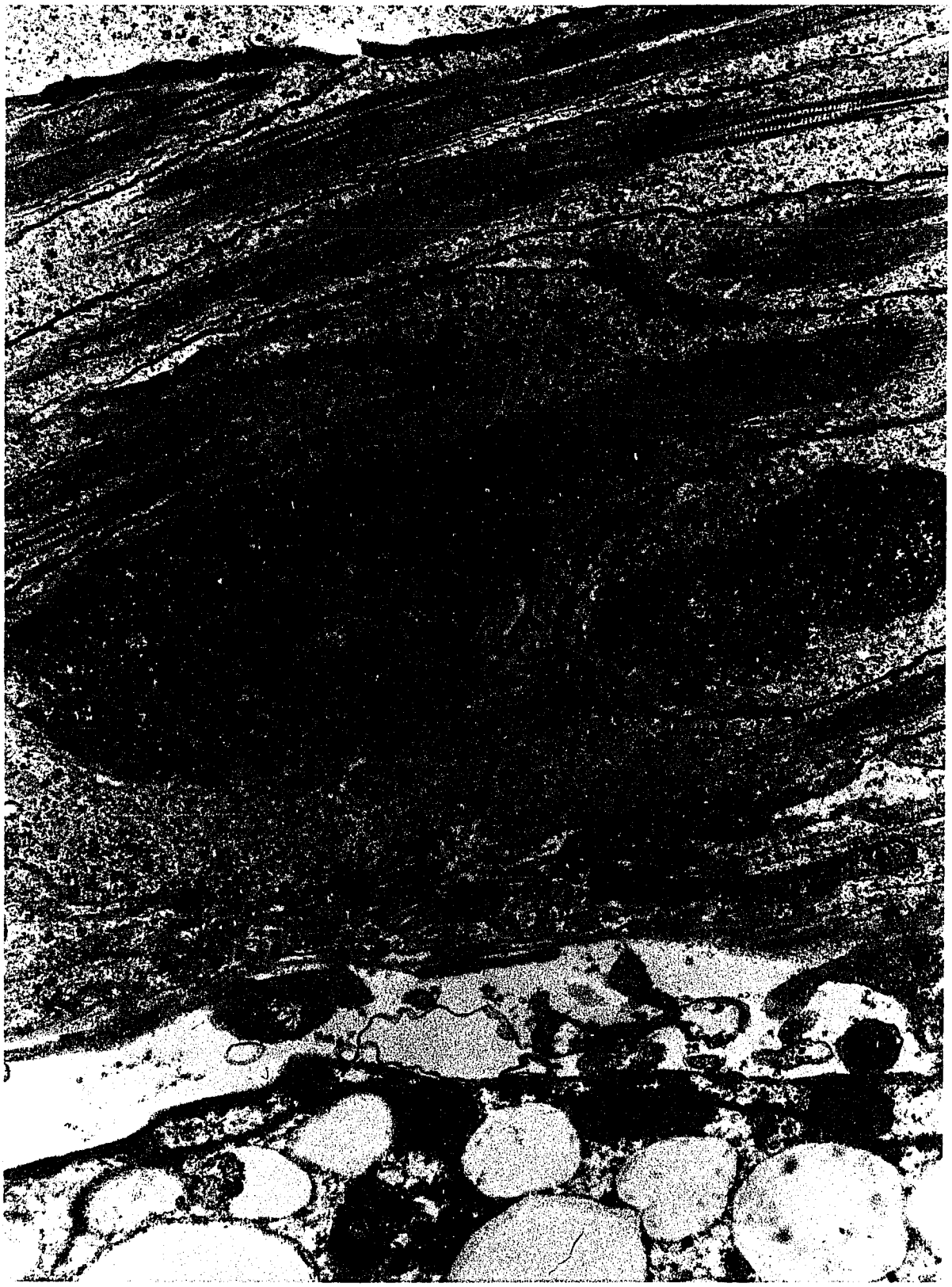

Figure 37. TEM section of Mexico hybrid testis. 22,000 X.

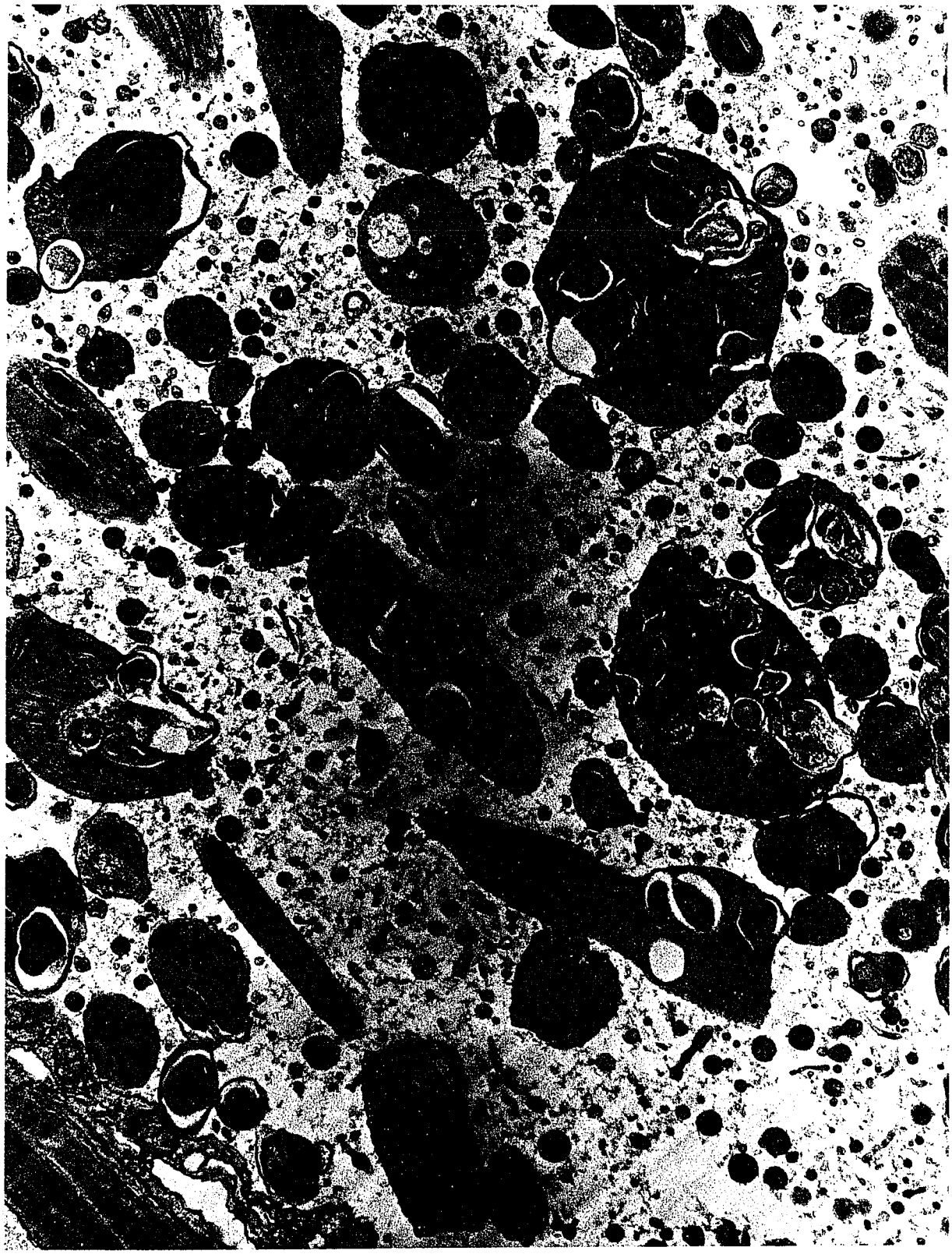

Figure 38. TEM section of Mexico hybrid testis. 19,000 X.

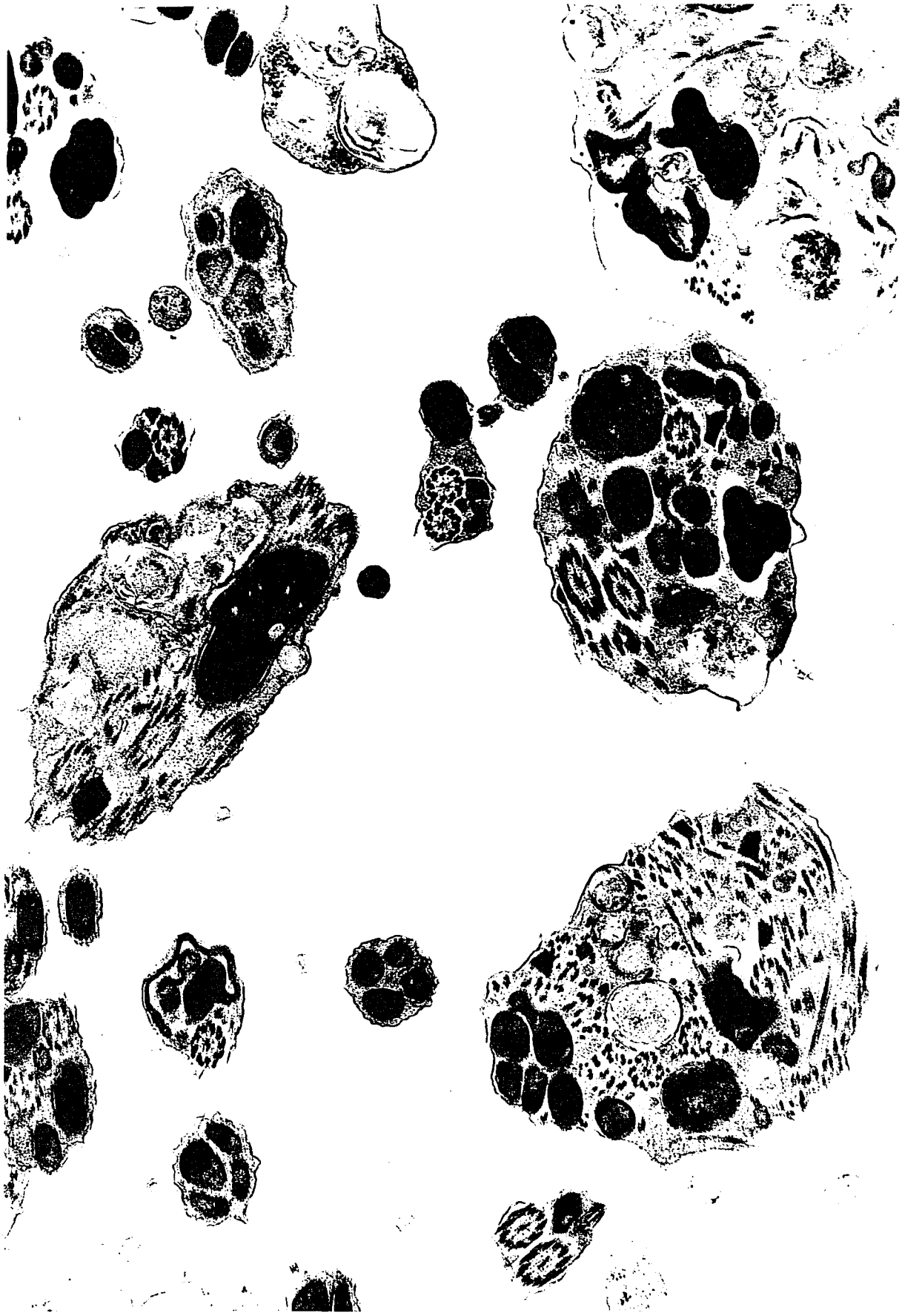

Figure 39. TEM section of Mexico hybrid testis. 19,000 X.

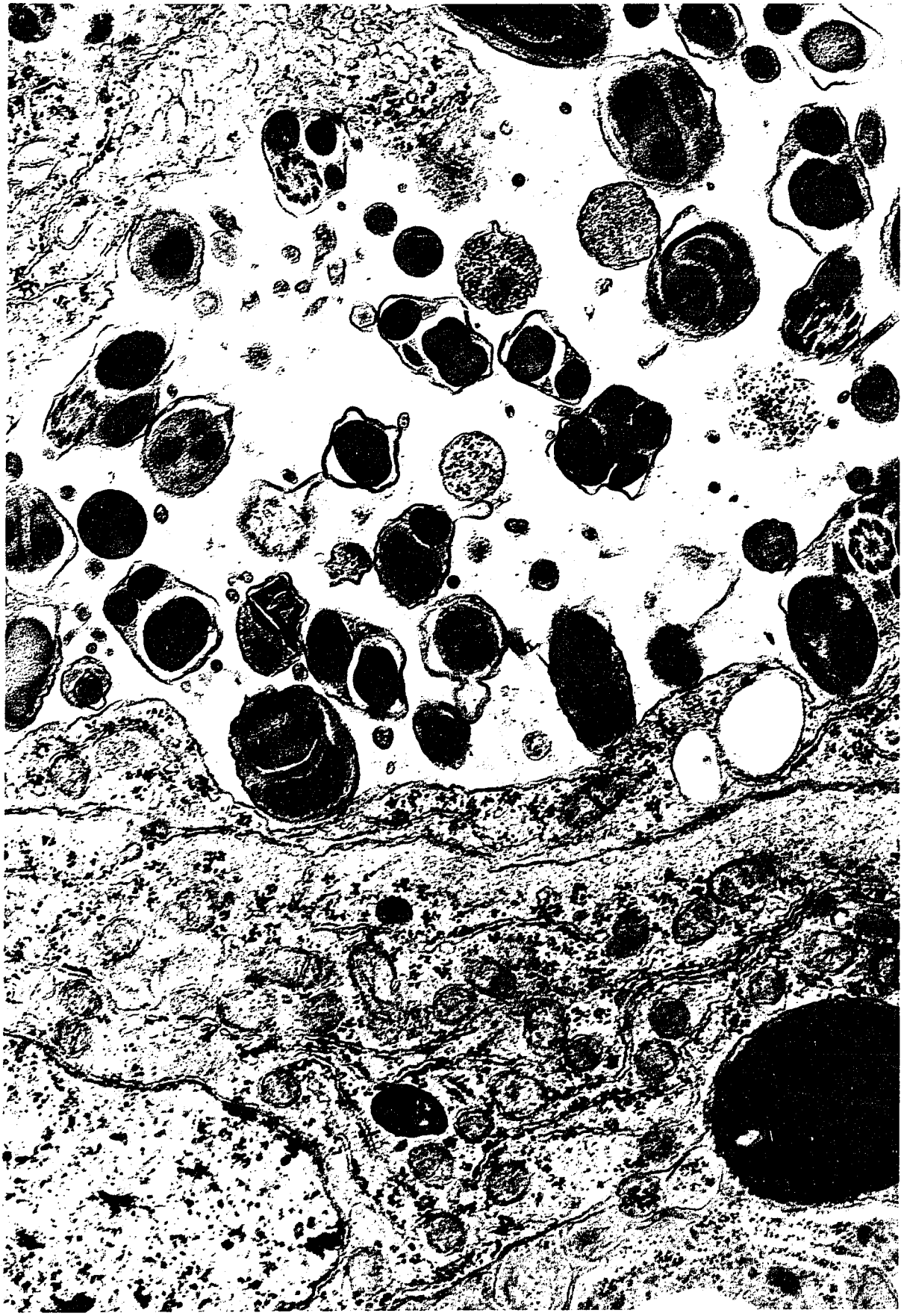

Figure 40. TEM section of Mexico hybrid testis. 29,000 X.

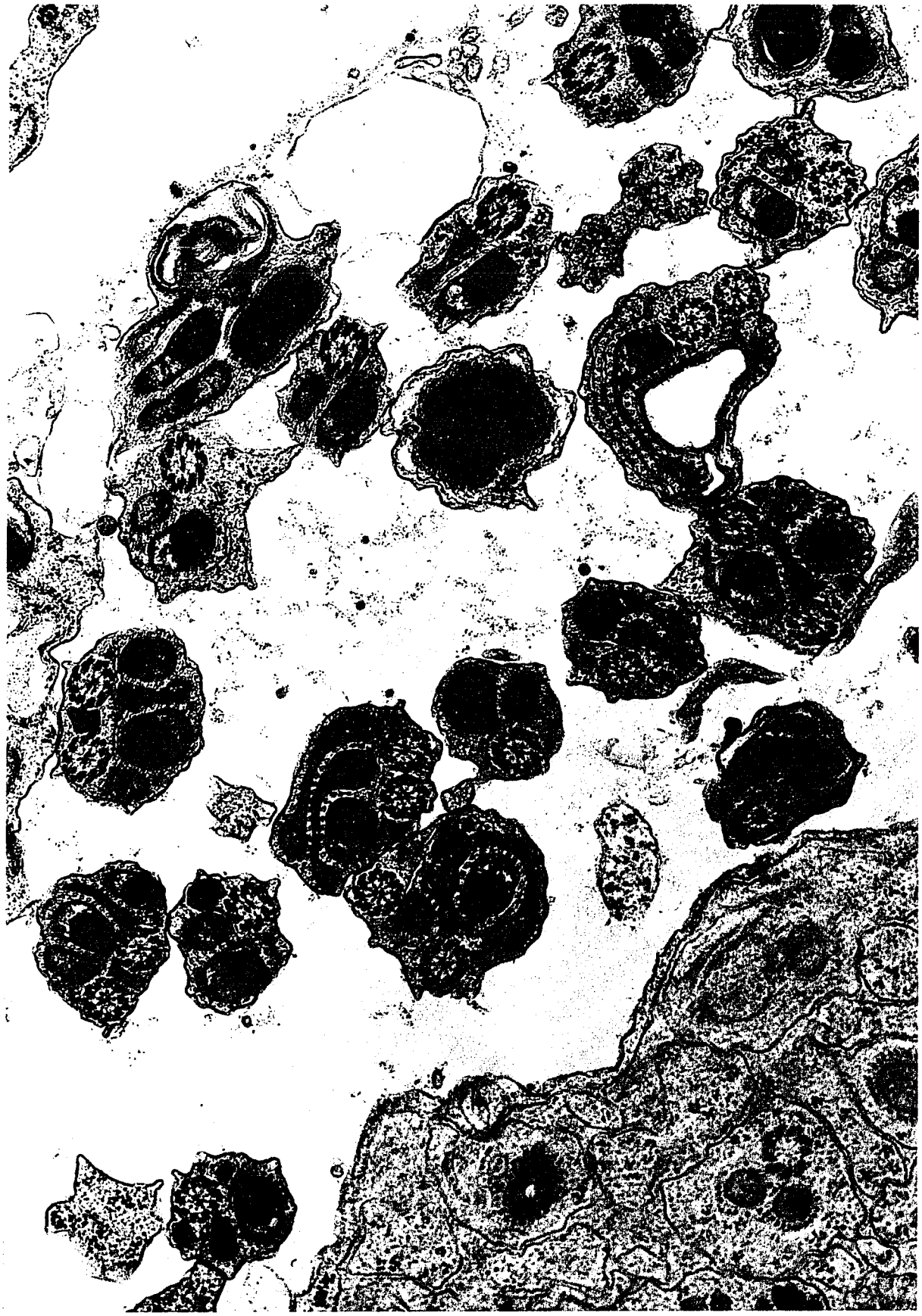

Figure 41. TEM section of Mexico hybrid testis. 40,000 X.

Figure 42. Male reproductive system of the boll weevil.

32 X.

APPENDIX

Phenomenon Observed	No Observations	No. Positive
1. Contraction of median oviduct in virgin females	10	10
2. Lack of streaming in spermathecal ducts of virgin females	10	10
3. Streaming in spermathecal duct of just mated females	8	8
4. Activation and attraction of sperm by spermathecal material	8	8
5. Activation of seminal vesicle sperm by male accessory gland material	9	9

VITA

Mary Laslie Grodner was born January 5, 1935, in Attapulgus, Georgia. After receiving an elementary education in Attapulgus she attended Gadsden County High School in Quincy, Florida, and was graduated in May, 1951. She received a Bachelor of Arts degree from Wesleyan College, Macon, Georgia, in June, 1955. She attended the Graduate School of Louisiana State University and received a Master of Science degree in Zoology in August, 1957. She then taught in the Department of Biology at Otterbein College in Westerville, Ohio. In 1959 she married Dr. Robert M. Grodner. After having two sons she re-entered the Graduate School of Louisiana State University. She is presently an instructor in the Department of Zoology and Physiology and is a candidate for the Doctor of Philosophy degree in Entomology.

EXAMINATION AND THESIS REPORT

Candidate: Mary Laslie Grodner

Major Field: Entomology

Title of Thesis: Aberrant Spermatogenesis in Hybrid Progeny of Sub-Species of the Boll Weevil (Coleoptera: Curculionidae)

Approved:

Norman W. Earle
Major Professor and Chairman

James D. Traynham
Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 18, 1973